Lipid and Fatty Acid Differences in Lake Trout (*Salvelinus namaycush*) Eggs from the Great Lakes, Cayuga Lake, and Lake Champlain

By

Robert Carl Louis Geroux

A thesis submitted to the Department of Environmental Science and Biology of The College at Brockport State University of New York in partial fulfillment of the requirements for the degree of Master of Science in Environmental Science and Biology

October 21, 2013

Lipid and Fatty Acid Differences in Lake Trout (*Salvelinus namaycush*) Eggs from the Great Lakes, Cayuga Lake, and Lake Champlain

Department of Environmental Science and Biology
Thesis Defense by

Robert C.L. Geroux

Date:

Master's Degree Advisory Committee	Approved	Not Approved
Major Advisor		
Committee Member		
Committee Member		

Graduate Coordinator Date

Chairman, Environmental Science & Biology Date

Acknowledgements

I would like to thank my major advisor Dr. Jacques Rinchard for his constant support, knowledge, and guidance throughout this project. From working in the aquatic lab to presenting my research at the 2011 American Fisheries Society meeting in Pittsburgh, he has given me many opportunities and experiences that will benefit me for the rest of my life.

I would like to thank my committee members Dr. Serguisz Czesny and Dr. Joseph Makarawicz who have given their time and effort in reviewing my thesis. They were an integral part of my college career, and it has been an honor to work with such talented scientists.

This project would not have been possible without the financial assistance provided by the Great Lakes Fisheries Trust. I would like to thank the following state, federal, and tribal agencies for their collection of fish samples: New York State Department of Environmental Conservation, Illinois Natural History Survey, US Fish and Wildlife Service (Green Bay office), Illinois Department of Natural Resources (DNR), Indiana DNR, Michigan DNR, Wisconsin DNR, Grand Traverse Bay of Ottawa and Chippewa Indians, Little River Band of Ottawa Indians, Chippewa Ottawa Resource Authority, the La Crosse Fish Health Center, and the Ontario Ministry of Natural Resources. I would like to thank Dr. Ellen Marsden for the Lake Champlain egg samples. My thanks to the faculty, staff, and students of The College at Brockport, Environmental Science and Biology Department: Dr. James Haynes, Blake Snyder, Ted Lewis, and Robert Cornish for their help collecting lake trout eggs from Lake Ontario.

Lastly, I would like to thank my friends and family for their continued love and support throughout this long process. I would specifically like to thank my Mom and Dad and my

wonderful fiancée Kristin, who pushed me to finish this endeavor; without their encouragement and support, none of this would be possible.

LIST OF TABLES

LIST OF FIGURES

Abstract

The main objectives of this study were to determine and compare fatty acid signatures (FAS) of lake trout eggs within and among the Great Lakes region. Fifteen sites were sampled over 2 years, including six sites in Lake Michigan, four sites in Lake Huron and one site each in Lake Ontario, Lake Superior, Lake Champlain, and Cayuga Lake. A total of 518 egg samples were quantified. A combination of univariate and multivariate statistical analyses was used to assess spatial and temporal differences in FAS in both the neutral lipid (NL) and phospholipid (PL) fractions of lake trout eggs. At each sampling site, FAS did not differ significantly between the 2 years of sampling. Therefore samples from 2009 and 2010 were combined to assess spatial differences. Discriminant factor analysis (DFA) was performed on lake trout eggs from 13 sample sites using 18 of the most abundant fatty acids detected. DFA revealed a clear separation of lake trout eggs by sample site reaching an overall classification success of 77.7% and 77.3% in the neutral lipid and phospholipid fractions, respectively. Similarly, nonmetric multidimensional scaling and SIMPER analyses revealed differences in FAS among sample sites in both lipid fractions. These differences were driven by 16:1n-7 and 18:1n-9 in the NL and by 16:0 and docosahexaenoic acid in the PL. We suggest that the differences observed in FAS in lake trout eggs among sample sites are reflective of the lake trout feeding habit.

1. Introduction

Lake trout *Salvelinus namaycush* is a salmonid species native to the Great Lakes, Finger Lakes, and Lake Champlain. Historically, lake trout flourished in their native habitats and were once the most valuable commercial fish in the Upper Great Lakes. Their populations declined in the 1950's due to combined pressures from overfishing, sea lamprey *Petromyzon marinus* predation, habitat degradation, and alewife *Alosa pseudoharengus* invasion (Hile *et al.* 1951, Eschmeyer 1955, Marsden and Langdon 2012). It has become a goal of many fisheries managers to restore and achieve self-sustaining lake trout populations in these water bodies (Bronte *et al.* 2008). This goal is achieved through catch limits, stocking efforts, and research. Currently, large numbers of lake trout are stocked in many of the Great Lakes, Finger Lakes, and Lake Champlain. According to the Great Lakes Fish Stocking Database (FWS/GLFC 2011) in 2011 total stocking efforts in the Great Lakes ranged from 221,885 fish stocked in Lake Erie to 3,454,179 fish stocked in Lake Michigan. According to the New York State Department of Environmental Conservation (Fish Stocking 2012), lake trout stocking in 2011 ranged from zero fish stocked in Cayuga Lake to 40,200 fish stocked in Seneca Lake. To date, efforts have not been successful outside of Lake Superior, although natural spawning has been observed in lakes Michigan and Huron (Riley *et al.* 2007, Bronte *et al.* 2008). Recently, Bronte *et al*. (2008) identified several factors affecting lake trout natural recruitment and impeding their rehabilitation efforts in Lake Michigan. These factors include early mortality syndrome (EMS, characterized by thiamine deficiency in lake trout alevins), predation on lake trout eggs and alevins, low adult populations, and improper stocking strategies.

Survival of lake trout offspring can be influenced by variation in egg quality. The term egg quality is often used loosely but can be defined as the ability of a gamete to be fertilized and

subsequently develop into a viable embryo (Bobe and Labbé 2010). Egg quality can be determined by the amount of proteins, carbohydrates, lipids, vitamins, hormones, and maternal mRNAs deposited into eggs by female fish during the process of vitellogenesis (Lubzens *et al*. 2010). Lipids, and their fatty acid components, have previously been used as a measure of egg quality (Sargent 1995, Czesny and Dabrowski 1998, Wiegand *et al.* 2004). There are two major fractions of lipids: neutral lipids (NL) and phospholipids (PL). Neutral lipids serve primarily as energetic reserves while phospholipids are used as the building blocks for biological membranes (Tocher 2003).

Lipids of both fractions are comprised of fatty acids. Structurally, fatty acids are carboxylic acids with a hydrocarbon chain. Fatty acids can be divided into saturated (SAFAs) and unsaturated fatty acids (Figure 1). Saturated fatty acids contain no double bonds while unsaturated fatty acids have at least one double bond in the hydrocarbon chain. Unsaturated fatty acids can further be classified as monounsaturated fatty acids (MUFAs), which contain a single double bond, and polyunsaturated fatty acids (PUFAs), which contain two or more double bonds. Fatty acids are designated in IUPAC (The International Union of Pure and Applied Chemistry) nomenclature by carbon chain length: number of double bonds and the position (n-x) of the first double bond with respect to the methyl end.

An important group of PUFAs are the essential fatty acids (EFAs) or fatty acids that cannot be synthesized by an organism and must be acquired through diet. The two EFAs required by most freshwater fish are linoleic acid (LA; 18:2n-6) and linolenic acid (ALA; 18:3n-3). These EFAs are needed to synthesize long chain fatty acids such as arachidonic acid (AA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3) (Sargent 1995, Tocher 2003). AA and EPA are both precursors to eicosanoids (Sargent 1995, Tocher 2003),

which play a critical role in inflammatory responses and are used as messenger molecules in the central nervous system (Tocher 2003). DHA is commonly found in phospholipid membranes of neural and retinal tissues (Tocher 2003).

Fatty acid profiles have been used to determine predator-prey relationships (Napolitano 1999, Dalsgaard *et al.* 2003). Lovern (1935) proposed that fatty acids are transferred from prey to predators and established the concept "you are what you eat." Fatty acids of carbon chain length greater than 14 are not broken down during the digestion process and are absorbed into the blood stream of monogastric animals (Smith *et al.* 1997). Since these fatty acids remain intact, it is possible to distinguish which fatty acids are synthesized by the predator and which are acquired through diet via prey (Iverson 1993). If diet determines which fatty acids are available to predators, then egg fatty acid composition may vary based on the fatty acids available to feeding female. Several laboratory studies have shown that maternal diet can influence lipid content and fatty acid profiles of fish eggs (Fernandez-Palacios *et al.* 1995, Navas *et al.* 1997, Rodriguez *et al.* 1998). Neutral fatty acid profiles tend to vary more than phospholipid profiles in response to manipulation of maternal diet (Wiegand 1996, Bell *et al.* 1997, Almansa *et al.* 1999). Variations in lipid and fatty acid profiles among wild fish populations have also been studied. Wiegand *et al.* (2004) compared lipid and fatty acid profiles of unfertilized walleye *Sander vitreus* eggs among ten sample sites in the Great Lakes and found that percent neutral lipids varied significantly among populations while percent phospholipids did not. Similarly, fatty acid profiles of neutral lipids differed significantly among populations, but fatty acid profiles of phospholipids did not. In another study conducted by Czesny and Dabrowski (1998), lipid and fatty acid profiles of wild caught and domesticated walleye eggs were analyzed and compared. These authors found that egg lipid concentrations and fatty acid

profiles differed among walleye populations. Linoleic acid concentration in the NL fraction was significantly higher in domesticated eggs while ARA, EPA, and DHA levels were higher in wild egg samples. Differences in the PL fraction were not as noticeable as differences in the NL fraction. Both studies revealed that lipids and fatty acids can vary by location and can be affected by the maternal diet. Czesny and Dabrowski (1998) also showed that PL were less affected by diet than NL.

There are several ways in which scientists use lipids and fatty acid to track trophic relationships among species. First, we can assume that individuals of the same species can alter, biosynthesize, and digest fatty acids in the same manner. Therefore if we find differences in fatty acid signatures among individuals from the same species, then we can assume that these differences occur because of dietary differences (Iverson *et al.* 2002). Secondly, we can use specific fatty acids as biomarkers. Fatty acid biomarkers are fatty acids that are only synthesized by certain organisms. For example, a study by Ackman and McLachlan (1977) found that 16:2n-4 and 16:4n-1 were only produced by certain diatoms. Thus, if these two fatty acids were found in higher tropic levels, it would be indicative of diatom consumption. Similarly, Czesny *et al*. (2011) found in Lake Michigan that pelagic fish presented higher concentrations of DHA , while benthic fish had higher levels of EPA and palmitoleic acid (16:1n-7). Thus the relative concentrations of fatty acids can indicate in what type of environment fish are foraging. Unfortunately, at higher trophic levels it becomes increasingly difficult to determine if the predator synthesized a particular fatty acid or if that organism in fact acquired that fatty acid in its diet. The third and most thorough approach has been to use the entire fatty acid profile to determine diet composition of predators as proposed by Iverson (1993). By knowing the fatty acid profile of a predator and all of its possible prey items, we can predict diet composition of a

predator based on its fatty acid profile and compare foraging differences within species and among systems (Budge *et al*. 2002, Iverson *et al*. 2002).

We know that prey assemblage differs among aquatic ecosystems. Alewife is the dominant prey item in Lake Michigan (Miller and Holey 1992, Madenjian *et al.* 1998, Madenjian *et al.* 2008), Lake Ontario (Bowlby *et al*. 2007), and Cayuga Lake (Bishop personal communication 2009). Historically, alewives were dominant in Lake Huron, but populations crashed in both 2004 and again 2007 after population increases between 2005 and 2006 (Schaeffer and O'Brien 2008). As of 2008, bloater *Coregonus hoyi* was the dominant prey in Lake Huron (Schaeffer and O'Brien 2008). In Lake Superior lake herring *Coregonus artedii* makes up a majority of the prey assemblage (Kitchell *et al.* 2000, Gorman *et al*. 2010). The most dominant prey item in Lake Erie is rainbow smelt *Osmerus mordax* followed by round goby *Neogobius melanostomus* (Lake Erie Forage Task Group 2012). In Lake Champlain, rainbow smelt, yellow perch *Perca flavescens*, and emerald shiners *Notropis atherinoides* make up the majority of the forage base although alewife abundance has increased since the early 2000's (Lake Champlain Fisheries Technical Committee 2009). One of the perquisites for the utility of fatty acid profiles as food web indicators is the assumption that each prey species has a distinct fatty acid signature. Although this assumption is well documented in marine environments (Budge *et al*. 2006), the utility of fatty acid signatures is not well documented in freshwater ecosystems. In a recent study, Czesny *et al*. (2011) reported that prey species collected in Lake Michigan had different amounts of mean lipid concentrations as well as different fatty acid signatures. Since prey assemblages differ among aquatic systems and female fish deposit lipids and fatty acids into their eggs we should be able to identify what female fish are eating based on the fatty acid profiles of their eggs.

Objectives

The main objective of this study was to compare fatty acid signatures (FAS) of lake trout eggs within and among the Great Lakes region (Lake Ontario, Lake Superior, Lake Michigan, Lake Huron, Lake Erie, Lake Champlain and Cayuga Lake). Specifically, I (1) determined the amount of total lipids (TL), neutral lipids (NL), and phospholipids (PL) in lake trout eggs from different locations; (2) determined the FAS of eggs from different locations; and (3) compared FA signatures among systems using a variety of univariate and multivariate techniques. In addition, I observed and compared the survival of lake trout embryos at two sample sites: Taughannock Falls (Cayuga Lake) and Hamlin Beach (Lake Ontario).

2. Materials and Methods

2.1. Site Selection, Sampling, and Egg Collection

Eggs of female lake trout were collected in lakes Ontario, Erie, Huron, Michigan, Superior, Champlain, and Cayuga Lake in fall 2009 and 2010. There were a total of thirteen sites sampled in 2009 and twelve in 2010 (Table 1 and Figure 2).

Because lake trout assessment surveys are conducted simultaneously by federal, state, and tribal agencies in the fall (mid-October through early November), I was not able to personally collect lake trout eggs from all my sample sites. However, the personnel of these agencies collected eggs as part of the thiamine biomonitoring conducted by the USGS-Great Lakes Science Center in collaboration with Dr. Rinchard (Table 1). Five to 10g of eggs was collected from each individual female and immediately frozen on dry ice prior to being shipped to Dr. Rinchard's laboratory at The College at Brockport - State University of New York. Upon

arrival, eggs were stored at -80°C until biochemical analysis. All samples were processed within 3 months of acquisition.

Nevertheless, I was able to collect eggs in two sites in New York: Hamlin Beach in Lake Ontario and Taughannock Falls in Cayuga Lake. At Hamlin Beach, vertical gill nets were set perpendicular to the shoreline over rocky reefs. Mesh size ranged from 1- to 6-inch stretch with each mesh panel being 50 feet in length. Thus, there was a 50-foot panel of 1-inch stretch mesh, followed by a 1.5-, 2-, 3-, 4-, 5-, and 6-inch stretch for a total net length of 350 feet. Soak time was approximately 18 h; nets were set at 4 pm and were pulled at 8 am the next morning. When nets were pulled, all lake trout and bycatch were removed. Ovulating female lake trout were stripped of eggs. Approximately 5 g of eggs was stored on ice and transported to The College at Brockport where they were stored at -80^{o}C for lipid and fatty acid analysis. The remaining eggs were directly fertilized on the boat using fresh sperm (see section 2.2.). Striped fish, as well as bycatch fish, were returned to the water. Green females were euthanized and taken back to laboratory. Individual fish were measured and weighed, and 5 g of ovarian tissue was collected and stored at -80°C for lipid and fatty acid analysis. Length (cm) and weight (kg) were taken in 2009 using a meter stick and hanging fish scale; whereas only length was taken in 2010 as there was a malfunction with the hanging fish scale.

I also participated in the egg take at Taughannock Falls, Cayuga Lake, which is conducted by the New York State Department of Environmental Conservation (NYSDEC) as part of their annual lake trout stocking program. The NYSDEC set and pulled all gill nets. Ovulating females and sperm producing males were stripped of eggs and sperm, respectively, by the NYSDEC. A subsample of eggs from individual females was taken and stored directly on ice, and approximately 200-300 eggs per female were fertilized (see section 2.2.). Upon arrival at

The College at Brockport, unfertilized egg samples were stored at -80°C for further biochemical analysis. Morphological data were not recorded.

2.2. Egg Fertilization, Incubation, and Embryo Survival

In addition to sampling eggs for lipid and fatty acid analysis, a portion of lake trout eggs collected from individual females at Hamlin and Taughannock Falls were fertilized to monitor embryo survival in 2009 and 2010. Eggs were fertilized using sperm from one to three male lake trout. Approximately 100µl of combined sperm was added to each portion of eggs (200-300 eggs). A small amount of water was added to the sperm/egg mixture to activate sperm. The sperm and egg mixture was then gently agitated for 1 minute. After mixing, eggs were rinsed three times with clean water to remove the excess sperm. Fertilized eggs were placed in plastic containers and filled with clean lake water. Containers were transported back to the laboratory at The College at Brockport. Water temperature was maintained by placing containers with eggs in a cooler with ice. A layer of newspaper was placed between the egg containers and ice to prevent eggs from coming in direct contact with ice.

Hatching baskets containing fertilized eggs were incubated in California-style hatching trays using recirculating water systems (Figure 3). Each basket contained fertilized eggs from a specific female fish. Hatching baskets were made from PVC pipe with a mesh bottom (Figure 4). Chillers (Frigid Units, Inc., Toledo, OH) were used to maintain water temperatures between 4°C and 10°C. The water source was charcoal filtered, dechlorinated, municipal water. Degree days were used to compare embryos at different developmental stages. Survival was evaluated at three different stages: pigmented eyed stage, hatching, and swim-up. Pigmented eyed stage is characterized by the appearance of pigments in the eye of the embryos. The hatching stage

occurs when embryos emerge from their eggs, whereas at the swim-up stage alevins have absorbed their yolk sac and start to swim in the water column. Water temperature and number of dead eggs were recorded daily during all developmental stages. Dead eggs and embryos were removed daily. Dead eggs turn white in color and can be easily identified and removed while dead embryos stop moving, turn translucent in color, and show signs of physical deterioration. At hatching, alevins were counted and transferred to 3-L, cylindrical aquaria with aeration (Figure 5). The number of alevins used to monitor survival to swim-up stage depended on the number of hatched alevins (40-400). Cylindrical aquaria were kept in a flow-through water bath to maintain constant water temperature. Water was added to the aquaria as needed to compensate for evaporative water loss. Particulate wastes (from dead eggs/embryos) were removed daily with a pipette. For each stage of development, the total number of surviving embryos/alevins from the previous stage was used at the start of the next stage of survival. For example, the total number of embryos that survived to the pigmented eyed stage was used as the starting number for the survival to the hatch stage. Similarly, the total number of hatched fish was the starting number for the swim-up survival study. Thus, survival was restarted at 100% survival at each stage of development.

2.3. Lipid Extraction

Total lipids were extracted from lake trout eggs following the gravimetric method originally proposed by Folch *et al.* (1957). One gram of unfertilized eggs/sample was placed in a homogenization tube. Twenty mL of 2:1 chloroform/methanol solvent with 0.01% butylated hydroxytoluene (BHT), used as antioxidant, was added to each homogenization tube containing samples. Tubes were then capped and placed on ice. Samples were homogenized for 1 minute

using an Omni homogenizer (Omni International, Kennesaw, GA). The homogenization tube was kept on ice for the entire process of homogenization. The Omni homogenizer probe was cleaned twice with deionized water and twice with the solvent between each sample homogenization. Next, homogenized samples were filtered under vacuum filtration using 11-μm Whatman filters (Whatman International Ltd., Piscataway, NJ) and transferred to large glass tubes. The homogenization tube was rinsed twice with solvent. The filtration flask was rinsed two times, with solvent, between each filtration to ensure that all lipids were transferred from the flask to the large glass tubes. After transfer, 4 mL of magnesium chloride ($MgCl_26H_2O$) was added to each sample. Large glass tubes containing samples were put under nitrogen, closed, and vortexed for one minute. Tubes were then refilled with nitrogen gas, capped, and stored at room temperature overnight.

After overnight storage, the bottom (solvent) layer containing the lipids was transferred to a new glass tube using a Pasteur pipette. The top (water) layer was discarded. Collected solvent was evaporated under nitrogen in a 30-35°C water bath. Samples were transferred to preweighed small glass tubes. The solvent was evaporated off of the samples, leaving only lipids. Total lipid amount in the sample was then determined gravimetrically. Finally a small aliquot of chloroform and nitrogen gas was added to the tube containing the lipid sample and stored at -80°C until lipid separation. Percent lipid content [(weight of lipid/weight of tissue)*100] was then calculated for each sample. All reusable glassware was cleaned using soap and water. Particulate matter was removed by scrubbing. Glassware was rinsed three times with tap water and three additional times with DI water before drying. Glassware was spot checked before using to make sure not debris or soap stains were present.

2.4. Neutral and Phospholipids Separation

Total lipids were separated into NL and PL according to the methods described by Juaneda and Roquelin (1985). Total lipids were eluted using a syringe with attached Sep-Pak silica columns (Waters Corporation, Milford, MA). First, total lipid samples were added to the column using a Pasteur pipette. Next, 20 mL of chloroform was added to the column to elute NL. Neutral lipids were collected in glass tubes. After collection of NL, the Sep-Pak silica column was moved over another glass tube and 20 mL of methanol was added to the column to elute the PL remaining in the column. Phospholipids were collected in glass tubes. Then each fraction was evaporated under nitrogen. Both the NL and PL fractions were transferred to separate, small, preweighed glass tubes and were evaporated under nitrogen. The amounts of NL and PL were determined gravimetrically. Percent NL was determined by dividing the amount of neutral lipid by the amount of total lipid and multiplying by one hundred. The same calculation was made for the PL fraction except that amount of PL was used in place of amount of neutral lipids.

2.5. Fatty Acid Transmethylation

Both NL and PL fractions were transmethylated following the methods described by Metcalfe and Schmitz (1961). Transmethylation replaces the hydroxide group, at the end of a fatty acid chain, with a methyl group. Attachment of the methyl group allows fatty acids to be identified using Gas Chromatography Mass Spectroscopy (GC/MS). A known amount of nonadocanoic acid (19:0) was added as an internal standard to each sample to calculate the amount of each fatty acid. After the addition of internal standard, samples were evaporated under nitrogen to remove excess solvent. Neutral lipids were then saponified using 1.5 mL of sodium hydroxide (NaOH 0.5 M in methanol) and incubated at 80ºC for 1 hour. Saponification is the

process by which an ester, in this case a triglyceride, undergoes nucleophilic acyl substitution producing a carboxylic acid. Essentially, saponification adds a hydroxide group to the neutral lipid. After incubation samples were cooled to room temperature, 2 mL of borontrifluoride methanol, which replaces the hydroxide group with a methyl group, was then added to each neutral lipid sample. Samples were placed under nitrogen and incubated at 80ºC for 30 minutes. After cooling to room temperature, 1 mL of hexane was added to each sample. Samples were capped and vortexed for 1 minute. Then, 1 mL of distilled water was added to each sample and vortexed again for 1 minute. The hexane phase was transferred to tubes containing anhydrous sodium sulfate, which absorbs water residues. An additional 1 mL of hexane was added to each sample, which was capped and vortexed. This additional hexane phase was transferred to the tubes containing anhydrous sodium sulfate. This double extraction with hexane ensures that all fatty acids are collected from samples. After transfer, the tubes containing the hexane phase and anhydrous sodium sulfate were vortexed. The hexane phase was transferred to 4-mL glass vials, put under nitrogen, capped, and stored at -80ºC until GC/MS analysis. The same procedure was used for PL, except for the saponification step which is not necessary because PL already contain a hydroxide group.

2.6. GC/MS Analysis

Fatty acids profiles were determined using an Agilent Technologies 7890A GC system with Agilent Technologies 7693 Autosampler and Agilent Technologies 5975C inert XL EI/CI MSD with Triple-Axis detector (Agilent Technologies, Inc., Santa Clara, CA). The capillary column is an Omegawax 250 Fused Silica Capillary Column with 30 m x 0.25 mm x 0.25 µm film thickness (Supelco, Bellefonte, PA). Helium was used as a carrier gas. The oven

temperature was programmed from 175^{o}C for 26 min to 205^{o}C at 2^{o}C/ min and then held at 205^{o}C for 24 min. The rate of helium carrier gas flow was 1.8 mL/min. The source and analyzer temperature of the MS was set at 230^{o}C. The individual fatty acid methyl esters (FAMEs) were identified by comparing the retention times of authentic standard mixtures (FAME mix 37 components, Supleco) and with known spectrographic patterns of FAMEs. Spectrographic patterns for FAMEs were acquired from the National Institute of Standards and Technology (NIST) Mass Spectral Library provided with the GC/MS and the American Oil Chemists' Society (AOCS) mass spectral library provided online at http://lipidlibrary.aocs.org/index.html. The FAMEs quantification was made by comparing individual fatty acid peak areas with that of the internal standard. Fatty acid concentrations are expressed as percent of total identified FAMEs.

2.7. Quality Control Procedures

As indicated previously (see 2.6.), FAMEs were identified by comparing their retention times with authentic standard mixtures and with known spectrographic patterns of FAMEs. Precautions were taken to account for retention time shifts. A running record of retention times were kept from previous injections. This allowed us to track shifts in retention time and accurately identify the correct peak of each FA over time. FA peaks were also manually checked to ensure that the software ChemStation properly identified and quantified them. If FA peaks were inaccurate, retention times were reexamined and adjusted to account for retention-time drift and peaks were reintegrated. A blank was run through the GC/MS after every 20 samples. Blank allows baseline to be adjusted to zero and to correct for background noise. The use of an auto injector ensured that samples were injected to the GC/MS similarly.

2.8. Statistical Analysis

Univariate and multivariate statistical analyses were performed. For univariate statistics, percentage data were arcsin transformed. Lipid data were checked for normality with a Shapiro-Wilk test and for homogeneity of variance with a Bartlett's test. Both the Shapiro-Wilk and Barlett's test failed, thus differences in lipid content (TL, NL, and PL) were tested using a Kruskal-Wallis test for both 2009 and 2010 data. Lipid data from 2009 and 2010 were then combined because the Kruskal-Wallis test revealed no differences in TL, NL, or PL concentration between years for the same sample site. Next, fatty acid data were checked for normality with a Shapiro-Wilk test and for homogeneity of variance with a Bartlett's test. FA data failed the Shapiro-Wilk and Bartlett's tests. Differences in FA concentrations were tested using a Kruskal-Wallis test in both the NL and PL fractions for 2009 and 2010 data. FA data from 2009 and 2010 were combined because the Kruskal-Wallis test revealed no differences in FA concentration between years for the same sample site. Since multiple comparisons were performed with the Kruskal-Wallis test, a Bonferroni correction was applied to the FA data. The Bonferroni correction decreases the alpha value thus decreasing the chance of a type one error. Since 24 different fatty acids were compared among 25 total sample sites, the alpha value (0.05) was divided by the number of fatty acids (24) to reduce the *p*-value to 0.002083 (0.05/24). Correlation analysis was used to determine correlations between fish length and FA concentration in lake trout eggs. All univariate analyses were performed in IBM SPSS 19.0 (SPSS Inc., Chicago Illinois).

Discriminant factor analysis (DFA), a multivariate technique, was used to compare FAS among sample sites in both lipid fractions. To prevent misclassification of subjects to groups

with the largest variance, equality of covariance matrices is required. To meet this requirement, the sample size of the smallest group must be larger than the number of predictor variables. This requirement was met by combining data from 2009 and 2010 to increase sample size. Consequently, I limited the number of fatty acids (predictor variables) to 18 (14:0, 16:0, 16:1n-9, 16:1n7, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 18:4n-3, 20:1, 20:4n-6, 20:4n-3, 20:5n-3, 22:4n-6, 22:5n-6, 22:5n-3, and 22:6n-3). Thus, all sample sites with sample size (*n*) of 19 or greater were included in the analysis. Sample sites included were Taughannock Falls, Hamlin Beach, Clay Banks, Drummond Island, Owen Sound, Portage Point, Waukegan, Old Mission, Michigan City, Grindstone, Milwaukee, Parry Sound, and Grand Isle. Sample sites Hamburg and Klondike Reef could not be included in the analysis because the number of samples was below 19 for both of these sample sites. Prior to DFA, percentage values were normalized using log-ratio transformed fatty acid data (Aitchison 1986) according to the equation: $X_{(trans)} = \ln (X_i/C_r)$ were X_i is a fatty acid expressed as percentage of total fatty acids, $X_{(trans)}$ is the transformed fatty acid data, and C_r is the geometric mean of all 18 fatty acid variables. Wilk's λ was used to test the significance of the DFA to separate groups. The number of observations correctly classified was used to evaluate the performance of the DFA. Classifications were cross-validated using a jack-knife procedure, which allowed me to determine into which group individuals were misclassified. DFA was conducted on IBM SPSS 19.0.

Nonparametric methods were also used to assess variation in FAS among sample sites in both lipid fractions. Nonparametric methods have no minimum sample size requirement, thus all data were included in the analysis. All nonparametric analyses were performed in PRIMER software v.6, (Primer-E Ltd., Plymouth, UK) using untransformed data of 24 fatty acids in both the NL and PL fractions expressed as percentage of total fatty acids (total NL in the NL fraction

and total PL in the PL fraction). First, the average value for each fatty acid was determined for all sample sites. For each lipid fraction, a Bray-Curtis resemblance matrix, which calculates the similarity between each sample pairing, was computed. A cluster analysis (CA) was then run to determine percent similarity (between 0 and 100 percent) among sample sites and to generate similarity contours. In addition to the CA, an analysis of similarity (ANOSIM) was performed to look at similarities between sample sites. The benefit of using ANOSIM is that it allows for the entire fatty acid signature to be used when comparing similarities between sample sites rather than just comparing individual fatty acids. ANOSIM uses an R-statistic to determine how similar two groups of data are to one another, in this case how similar fatty acid signatures from two samples sites are. R-statistic of 0.0 indicates that within group samples are no more similar to one another than they are to samples from another group. R-statistic of 1.0 indicates that within group samples are more similar to one another than they are to samples from another sample site. Cluster analysis and ANOSIM were generated for FAS in both the NL and PL fraction.

Nonmetric multidimensional scaling (nMDS) plots with overlain similarity contours were generated to visualize the amount of similarity (or dissimilarity) among sample sites. The relative level of distortion in each nMDS plot is described by its stress level. Stress levels less than 0.1 indicate accurate representation of data. Stress levels above 0.2 may be inaccurate and caution should be used when interpreting. Cluster analysis was used to select percent similarity contours. Bubble plots were overlain on the similarity contours to show relative abundance of selected FA. In addition to nMDS, a similarity percentages (SIMPER) routine was conducted to evaluate similarity and/or dissimilarity among sample sites. SIMPER determines the similarity and dissimilarity between sample sites. In addition, SIMPER identifies individual fatty acids

responsible for the observed similarity and dissimilarity. Finally, a principle component analysis (PCA) was used to help visualize the results from the SIMPER routines.

For the analysis of embryo survival, all percentage data were arcsine transformed. Normality of the data was tested using a Shapiro-Wilk test; whereas the homogeneity of variance was tested using a Bartlett's test. Since data were not normal, a Mann-Whitney test was used to determine differences in survival between Taughannock Falls and Hamlin Beach for both 2009 and 2010 data. The Mann-Whitney tests were performed on IBM SPSS 19. A Pearson Coefficient (R) was used to determine the strength and direction of the relationship between embryo survival and fatty acid concentration with an R value of 1.0 indicating a strong correlation and an R value of 0 indicating no correlation. Correlations were performed on IBM SPSS 19.

3. Results

3.1. Egg Collection and Fish Morphology

Variability in catch rates resulted in incomplete sampling between years. In 2009, lakes Champlain and Erie were not sampled. In 2010, Grindstone and Owen Sound in Lake Huron and Klondike Reef in Lake Superior were not sampled. A total of 518 lake trout egg samples from the Great Lakes, Cayuga Lake, and Lake Champlain were collected between 2009 and 2010 and analyzed for total lipid and fatty acid composition. Table 1 shows the total number of fish caught at each sample site, as well as fish length and weight. Some length and weight data were not recorded by collection agencies. Fish from all sample sites were similar in length and weight, except for fish from Lake Superior which were smaller. Significant correlations between female fish length and the concentration of individual FA were determined and are presented in Table 2.

3.2. General Trends in Total Lipids, Neutral Lipids, and Phospholipids

Total, neutral, and phospho-lipid concentrations in lake trout eggs collected in 2009 and 2010 are presented in Table 3. Statistical analyses revealed no year effect in TL, NL, PL, and fatty acid concentrations among populations between 2009 and 2010 at each sample site. Since there was no year effect, samples collected in 2009 and 2010 were combined to form one set of data for each sample location. After combining data, statistical differences among locations were observed in total lipids of lake trout eggs (Kruskal-Wallis, Chi-square = 136.129, df = 14, $p < 0.05$). The concentration of total lipid was statistically highest in eggs from Clay Banks and lowest in Parry Sound and Grand Isle (Tamhane's *post hoc*, $p < 0.001$) (Table 3). In general, Hamburg (L. Erie), Hamlin Beach (L. Ontario), and Taughannock Falls (Cayuga Lake) had similar levels of TL compared to all sites from Lake Michigan. All sites from Lake Huron had similar amounts of TL and were always lower than sites from Lake Michigan. Statistical differences among locations were also observed in the lake trout egg NL and PL concentrations (Kruskal-Wallis, Chi-square = 66.126, df = 14, $p < 0.001$ and Kruskal-Walis, Chi-square = 66.146, df = 14, $p < 0.001$ for NL and PL, respectively). The concentration of NL was highest in eggs from Hamburg and lowest in Klondike Reef. The percentage of NL was fairly consistent among sample sites except for Klondike Reef which had a significantly lower percentage of NL than the rest of the sample sites (Tamhane's *post hoc*, $p < 0.001$) (Table 3). The concentration of PL showed the opposite trend.

3.3. General Trends in Fatty Acid Signatures

There were a total of 24 fatty acids routinely identified in both the neutral and phospholipid fractions. In both lipid fractions, SAFAs were dominated by palmitic acid (16:0) and myristic acid (14:0), whereas MUFAs were dominated by palmitoleic acid (16:1n-7) and oleic acid (18:1n-9). Docosahexaenoic acid, EPA, ARA, linoleic acid, and linolenic acid were the most abundant PUFAs detected in both the neutral and phospholipid fractions. Although both the NL and PL fractions were dominated by the same fatty acids, there were noticeable differences in the concentrations of those fatty acids between fractions. The NL fraction contained less SAFA than the PL fraction. Higher concentrations of 14:0 were observed in the NL fraction than the PL fraction, whereas the lower concentrations of 16:0 and 18:4n-3 were observed in the NL fraction compared to the PL fraction. The NL fraction contained about twice the concentration of MUFAs than the PL fraction. The concentration of palmitoleic acid was four times greater in the NL than in the PL fraction. Similarly, the concentration of oleic acid was more than twice as high in the NL fraction compared to the PL fraction. The total concentration of *n*-6 PUFA was similar in both the NL and PL fraction although there were noticeable differences in the concentrations of specific PUFA. In the NL fraction the major *n*-6 PUFA detected were 18:3n-3 and ARA, while ARA was the single largest contributor in the PL fraction. Arachidonic acid had a higher concentration in the PL fraction compared to the NL fraction, while 18:2n-6 was lower in the PL fraction compared to the NL fraction. The concentration of ALA was much less in the PL fraction than in the NL fraction. The total concentration of *n*-3 PUFA was higher in the PL fraction than in the NL fraction. Eicosapentaenoic acid concentrations were similar in the NL and PL fractions. The PL fraction contained more than twice the amount of DHA than the NL fraction.

3.4. Among Lake Differences in Fatty Acids in the Neutral Lipid Fraction

Fatty acids from the NL fraction differed among sample sites; however, sample sites within the same lake system showed less variation. Significant differences were observed in the concentration of SAFA (Kruskal-Walis, Chi-square = 135.312, df = 14, $p < 0.001$) among all sample sites. Eggs from Taughannock Falls had the statistically highest concentration of SAFA, while eggs form Owen Sound had the lowest concentration (BFC $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). The highest concentration of 16:0 was observed in eggs collected in Grindstone and the lowest in the ones from Owen Sound (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). The highest observed concentration of 14:0 was observed in the eggs from Taughannock Falls while the lowest was found in eggs from Grindstone (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). The highest percent concentration of 18:0 was observed in Parry Sound and lowest in Klondike Reef (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) (Table 4).

Significant differences were observed in MUFA concentration (Kruskal-Wallis, Chi-square = 147.972, df = 14, $p < 0.001$) among all sample sites. Eggs from Grindstone had the highest concentrations of MUFA and eggs from Portage Point had the lowest concentration of MUFA (BFC $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). The highest concentration of 18:1n-9 was in fish eggs from Parry Sound and lowest in eggs from Portage Point (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). The highest concentration of 16:1n-7 was found in fish eggs from Hamburg and lowest in eggs from Taughannock Falls (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). The third most detected MUFA was 18:1n-7 (vaccenic acid). The concentration of 18:1n-7 was statistically highest in Grindstone and lowest in Taughannock Falls (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) (Table 4).

The sum of PUFA was statistically different (Kruskal-Wallis, Chi-square = 131.619, df = 14, $p < 0.001$) among all sample sites. The percentage of PUFA was statistically highest (BFC $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) in lake trout eggs from Portage Point and lowest in those from Grindstone (BFC $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). The sum of *n*-6 PUFA was statistically different in lake trout eggs among sample sites (Kruskal-Wallis, Chi-square = 188.466, df = 14, $p < 0.001$). The sum of *n*-6 was statistically highest in eggs from Portage Point and lowest in eggs from Hamburg (BFC $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). The concentration of linoleic acid was statistically highest in Klondike Reef and lowest in Owen Sound (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). The highest concentration of ARA was observed in Taughannock Falls while Klondike Reef had the lowest concentration of ARA (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) (Table 4).

The sum of *n*-3 PUFA was statistically different in lake trout eggs among sample sites (Kruskal-Wallis, Chi-square = 112.058, df = 14, $p < 0.001$). Portage Point had the highest concentration of *n*-3 PUFA while Grindstone had the lowest concentration of *n*-3 PUFA. These results were statistically significant (BFC $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$). The two dominant *n*-3 PUFA in the NL fraction were DHA and EPA followed by 22:5n-3 and linolenic Acid. The highest concentration of DHA was found in fish from Portage Point, while fish from Grand Isle had the lowest concentration (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). The statistically highest concentration of EPA was found in Klondike Reef while the lowest concentration was found in lake trout eggs from Grand Isle (BFC $\alpha = 0.002$, Tamhane's *post hoc* $p < 0.001$) (Table 4). Waukegan had the statistically highest concentration of 22:5n-3 and Grand Isle had the lowest (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). The highest

concentration of 18:3n-3 was found in eggs from Grand Isle and the lowest concentration in eggs from Owen Sound (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) (Table 4).

The ratio of $\Sigma n\text{-}3/\Sigma n\text{-}6$ was statistically highest in Grand Isle and Hamburg and lowest in Hamlin Beach, Michigan City, and Grindstone (BFC $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$). However, the ratio of $\Sigma n\text{-}3/\Sigma n\text{-}6$ in Grand Isle and Hamburg were not statistically different from each other. Likewise, the ratio of $\Sigma n\text{-}3/\Sigma n\text{-}6$ in Hamlin Beach, Michigan City, and Grindstone were not statistically different from each other. The ratio of DHA/EPA was statistically highest in Parry Sound and lowest in Hamlin Beach and Hamburg (BFC $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). There was little variation in the ratio of ARA to EPA among sample sites. Taughannock Falls, Clay Banks, Milwaukee, Michigan City, and Portage Point all had a concentration of $0.7 \pm 0.1\%$, which was statistically higher than the ratio of ARA to EPA in eggs from Klondike reef (BFC $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) (Table 4). The ratio of ARA to EPA in Taughannock Falls, Clay Banks, Milwaukee, Michigan City, and Portage Point was not statistically different (Table 4).

3.5. Among Lake Differences in Fatty Acids in the Phospholipid Fraction

As in the NL fraction, there were differences among sample sites in the FA of lake trout eggs in the PL fraction. The sum of SAFA was statistically significant among sample sites (Kruskal-Wallis, Chi-square = 150.637, df = 14, $p < 0.001$). The percentage of SAFA was statistically highest (BFC $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) in lake trout eggs from Parry Sound and Hamburg and lowest in lake trout eggs from Waukegan (Table 5). The concentration of palmitic acid was statistically highest (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) in Old Mission, Parry Sound, and Grand Isle and lowest in Waukegan (Table 5). The

concentration of 18:0 was statistically highest (BFC α = 0.002, Tamhane's *post hoc*, $p < 0.001$) in Parry Sound and Hamburg and lowest in Klondike Reef (Table 5).

The sum of MUFA was statistically different (Kruskal-Wallis, Chi-square = 110.574, df = 14, $p < 0.001$) among all sample sites. The percentage of MUFA was statistically highest (BFC α = 0.006, Tamhane's *post hoc*, $p < 0.001$) in lake trout eggs from Owen Sound and Hamburg and lowest in eggs from Taughannock Falls (Table 5). The concentration of oleic acid was statistically highest (BFC α = 0.002, Tamhane's *post hoc*, $p < 0.001$) in Owen Sound and Grand Isle and lowest in Klondike Reef (Table 4). The concentration of 18:1n-7 was highest (BFC α = 0.002, Tamhane's *post hoc*, $p < 0.001$) in Hamburg and lowest in Taughannock Falls (Table 5).

The sum of PUFA was statistically different (Kruskal-Wallis, Chi-square = 152.272, df = 14, $p < 0.001$) among all sample sites. The percentage of PUFA was statistically highest (BFC α = 0.006, Tamhane's *post hoc*, $p < 0.001$) in lake trout eggs from Klondike Reef and lowest in eggs from Hamburg (Table 5). The sum of *n*-6 was statistically highest (BFC α = 0.006, Tamhane's *post hoc*, $p < 0.001$) in eggs from Taughannock Falls and lowest in eggs from Hamburg (Table 5). The statistically highest (BFC α = 0.002, Tamhane's *post hoc*, $p < 0.001$) concentration of ARA was found in lake trout eggs from Taughannock Falls and the lowest in eggs from Klondike Reef (Table 5). The highest concentration of linoleic acid (BFC α = 0.002, Tamhane's *post hoc*, $p < 0.001$) was found in eggs from Taughannock Falls, Hamlin Beach, and Klondike Reef while Owen Sound had the lowest concentration of linoleic acid (Table 5). The sum of *n*-3 was statistically highest (BFC α = 0.006, Tamhane's *post hoc*, $p < 0.001$) in lake trout eggs from Klondike Reef and lowest in eggs from Hamburg (Table 5). Docosahexaenoic acid has the most dominant *n*-3 fatty acid followed by EPA. The statistically highest concentration (BFC α = 0.002, Tamhane's *post hoc*, $p < 0.001$) of DHA was found in eggs from Klondike Reef

and the lowest concentration was in eggs from Hamburg (Table 5). Lake trout eggs from Klondike Reef had the statistically highest concentration (BFC $\alpha = 0.002$, Tamhane's *post hoc*, $p < 0.001$) of EPA while eggs from Owen Sound and Parry Sound had the lowest concentrations (Table 5).

The ratio of *n*-3/*n*-6 in lake trout eggs was statistically different among sample sites (Kruskal-Wallis, Chi-square = 235.542, df = 14, $p < 0.001$). Eggs from Hamburg and Klondike Reef had the statistically highest *n*-3/*n*-6 ratio (BFC $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) while eggs from Taughannock Falls had the lowest *n*-3/*n*-6 ratio (Table 5). There was a statistically significant difference (Kruskal-Wallis, Chi-square = 205.208, df = 14, $p < 0.001$) in the ratio of DHA to EPA in lake trout eggs among sample sites. Owen Sound had the statistically highest (BFC $\alpha = 0.006$, Tamhane's *post hoc*, $p < 0.001$) ratio of DHA to EPA and Hamburg had the lowest ratio of DHA to EPA (Table 3). There was little variation in the ratio of ARA to EPA in lake trout eggs among sample sites; however, this difference was statistically significant (Kruskal-Wallis, Chi-square = 175.201, df = 14, $p < 0.001$). Eggs from Klondike Reef had the lowest ration of ARA to EPA (Table 5).

3.6. Comparison of FAS Using Discriminant Factor Analysis

DFA was performed on fish eggs from 13 samples sites (with $n \geq 18$) using the 18 most abundant fatty acids detected. DFA revealed separation of groups by lake and sample site in both the neutral lipid (Figure 6) and phospholipid (Figure 7) fractions. There were a total of twelve discriminant functions (DFs), or linear combinations of variables, generated by DFA in both the neutral lipid and phospholipid fractions.

In the DFA using FAS from the NL fraction, the first two DFs accounted for 62.4% percent of the variance (DF1= 48.9 % and DF2 = 13.5%) in fatty acid composition among samples. Inclusion of DF3 (11.8%) and DF4 (7.8%) increased cumulative variation explained to 81.9%. DF1 was defined positively by 18:1n-7 and negatively by 14:0. DF2 was characterized by positive loadings of 20:4n-6, 22:4n-6, and 22:5n-6 and negative loading of 18:1n-9. The third discriminant function was defined positively by 16:1n-9 and negatively by 18:3n-3 and 18:4n-3. The fourth discriminant function defined positively by 20:4n-3 and 18:2n-6 and negatively by 18:0. A bivariate plot of only DF1 and DF2 showed a clear separation of samples by lake (Figure 6). DFA correctly classified 77.7% of samples (Wilks'$\lambda = 0.003$; $P < 0.0001$). Cross-validation using jackknife procedure yielded a 71.9% overall success rate in predicted group membership. In general, misclassified egg samples (egg samples not classified from the correct sample site) were from Lake Michigan or Lake Huron. For example, of the 47 eggs sampled from Drummond 25 were misclassified. Misclassified eggs were grouped with eggs from other sample sites in Lake Huron and Lake Michigan but not from any other lakes. In addition, some sample sites, such as Taughannock Falls and Grand Isle, had no misclassified eggs.

In the DFA using FAS from the PL fraction, the first two DFs accounted for 63.7% percent of the variance (DF1= 49.7 % and DF2 = 14.1%) in fatty acid composition among samples. Inclusion of DF3 (9.5%) and DF4 (7.4%) increased cumulative variation explained to 80.6%. DF1 was defined negatively by 18:1n-7. DF2 defined positively by 16:1n-9 and 16:0. DF2 was negatively defined by 18:2n-6, 20:4n-3, 22:4n-6, 18:4n-3, and 18:3n-3. DF 3 was positively defined by 16:1n-7; DF 3 was negatively defined by 20:4n-6 and 22:5n-6. The fourth discriminant function was defined positively by 20:1. A bivariate plot of only DF1 and DF2 showed a clear separation of samples by lake (Figure 7). DFA correctly classified 77.3% of

samples (Wilks'$\lambda = 0.004$; $P < 0.0001$). Cross-validation using jackknife procedure yielded a overall success rate of 70.7%. As with the NL fraction, the majority of misclassified egg samples came from lakes Michigan and Huron sample sites. Similarly, Taughannock Falls and Grand Isle samples were not misclassified.

3.7. Comparison of FAS Using Analysis of Similarity, Nonmetric Multidimensional Scaling, and Principal Component Analysis

Similarities in FAS among sample sites are illustrated in an nMDS plot with overlain similarity contours, for both the NL and PL fractions (Figures 8 and 9, respectively). For both NL and PL fractions the stress level was 0.15. In the NL fraction, clusters were formed at the 90%, 95%, and 97% similarity level (Figure 8). All sample sites formed a single cluster at the 90% similarity level. Four clusters emerged at the 95% similarity level. One cluster contained Taughannock Falls and Hamlin Beach, another contained Parry Sound and Owen Sound, a third cluster contained Grindstone and Hamburg, and lastly a fourth cluster encompassed Old Mission, Portage Point, Klondike Reef, Drummond Island, Milwaukee, Clay Banks, Waukegan, and Michigan City. A single cluster emerged at the 97% similarity level. It encompassed Milwaukee, Clay Banks, Waukegan, and Michigan City. In the PL fraction, clusters were formed at the 90%, 95% and 97% similarity level (Figure 9). In the PL fraction, all sample sites formed a single cluster at the 90% similarity level. At the 95% similarity level a single cluster emerged incorporating all sample sites except for Klondike Reef and Hamburg. At the 97% similarity level three clusters emerged. The first cluster contained Michigan and Waukegan, the second contained Parry Sound and Owen Sound, and finally the third cluster encompassed Milwaukee, Clay Banks, Old Mission, and Portage Point. ANOSIM revealed differences in FAS among

sample sites in both the NL and PL fractions (Tables 6 and 7, respectively) as well. In both the NL and PL fractions, PCA reduced 24 fatty acids into five principal components. In the NL fraction the first two principal components explained 78.8% of the variance in the data set (Figure 10). Variance in principal component 1 (PC1) was explained by 18:1n-9. Variance in principal component 2 (PC2) was explained by 16:1n-7 and 18:1n-7. In the PL fraction the first two principal components explained 58.5% of the variance in data (Figure 11). Variance in principal component 1 (PC1) was explained by 22:6n-3. Variance in principal component 2 (PC2) was explained by 16:0 and was contributed to negatively by 20:4n-6.

SIMPER routines revealed within site similarity and among site dissimilarity in FAS. In the NL fraction within site similarity ranged from 91-96% similarity. Three FA, 18:1n-9, DHA, and 16:0, combined to explain 40% of the within-site similarity at each sample site. Dissimilarity among sample sites ranged from 7-13% dissimilarity (Table 8). This dissimilarity was caused by 18:1n-9, 16:1n-7, and 18:1n-7. In the PL fraction within site similarity ranged from 92-96% similar. The two main fatty acids that caused this similarity were DHA and 16:0, which combined to explain 40% of the within-site similarity. Dissimilarity among sample sites ranged from 5-9% dissimilarity (Table 9). This dissimilarity between sample sites was caused mostly by DHA, 16:0, and 20:4n-6.

3.8. Survival of Lake Trout Embryos from Lake Ontario and Cayuga Lake

There were a total of 24 and 26 fish sampled from Taughannock Falls in 2009 and 2010, respectively. All fish sampled from Taughannock Falls (2009 and 2010) were used in the survival study. Of the 12 fish sampled in 2009 and the 27 fish sampled in 2010, only five fish were used in the survival study from Hamlin Beach in 2009 and three fish from 2010 (Table 10).

Sample sizes are lower in the survival analysis because green females were used in fatty acid analysis but not the survival analysis. Survival to pigmented-eyed stage was not statistically different between sample sites in 2009 (U = 41.0, df = 27, $p > 0.05$). Survival at hatching was statistically higher in Taughannock Falls than in Hamlin Beach in 2009 (U = 120.0, df =27, $p < 0.05$). Survival at the swim-up stage between Taughannock Falls and Hamlin Beach in 2009 did not differ significantly (U = 71.0, df = 27, $p > 0.05$). There were no statistical differences between survival to pigmented-eyed stage (U=53.0, df = 27, $p > 0.05$), hatching (U = 34.0, df = 27, $p > 0.05$), or swim-up (U = 63.0, df = 27, $p > 0.05$) between Taughannock Falls and Hamlin Beach in 2010.

Correlations between survival of lake trout embryo and specific fatty acids in both the NL and PL fractions were investigated. Significant correlations were found and are presented in Table 11 and Figures 12 and 13.

4. Discussion

Our research represents the first study to simultaneously compare lipids and fatty acids both temporally and spatially in lake trout eggs. Interestingly, our study found no differences in TL, NL, PL, or FA concentrations temporally. It is possible that a larger data set, spanning more than 2 years, would show temporal variation in lake trout eggs. However, the lack of temporal variation allowed us to combine data from fish collected in 2009 and 2010 and helped to provide a better understanding of lipid and fatty acids differences spatially, where we were able to identify differences.

Lake trout eggs from all sample sites were rich in lipids, regardless of origin. In freshwater fish species, ripe fish eggs on average ranged from 2% to 10% lipids based on wet

weight. It was reported that yellow perch contained less than 5% total lipids (Kaitaranta and Ackman 1981), whereas common whitefish *Coregonus lavaretus* contained 11.5% total lipids (Kaitaranta 1980). Total lipids in lake trout ova from this study ranged from 8.8 – 9.5% of wet weight. These levels are consistent with findings in other studies. Czesny *et al.* (2012) found that lake trout eggs from two sites in Lake Michigan (Algoma and Waukegan) had total lipid content (Mean ± SD) of 9.9 ± 0.6% (Algoma) and 9.1 ± 0.7% (Waukegan). Since it takes lake trout embryos several months to develop and food is not readily available after hatching, total lipid reserves are very important to embryo survival and must be high enough to sustain embryos during their development.

In our study, TL comprised of 50.7-56.7% NL and 43.4-49.3% PL. This is consistent with other studies conducted in Lake Michigan by Czesny *et al*. (2009, 2012). In terms of spatial differences in specific FA, Czesny *et al*. (2012) found that DHA concentration differed between Algoma and Waukegan in both the NL and PL fraction. Likewise, the concentrations of FA detected by Czesny *et al*. (2012), in both the NL and PL fractions, were similar to levels detected in our study.

FAS have been used to identify predator-prey relationships (Dalsgaard *et al.* 2003, Falk-Petersen *et al*. 2004) following the concept "you are what you eat." Since fatty acids with carbon chain length greater than 14 remain intact during digestion in monogastric animals (Smith *et al*. 1997), it is possible to distinguish which fatty acids are acquired through diet and which are synthesized by the predator (Iverson 1993). In a recent study by McKenna *et al*. (unpublished data), food web composition (based on gut contents of lake trout and FAS) was shown to vary both within a lake system and among lake systems. Specifically, lake trout in Lake Michigan fed mostly of alewives on the western side of the lake, while fish on the eastern shore of Lake

Michigan fed on both alewives and round gobies. McKenna *et al*. (unpublished data) also found that lake trout in Lake Huron fed mostly on rainbow smelt and round goby. In another study, Czesny *et al.* (2011) found that alewives from Lake Michigan had high levels of DHA (15.5%) and low concentrations of 16:1n-7 (4.7%) compared to round goby which had a DHA concentration of 6.8% and a 16:1n-7 concentration of 9.5%. Knowing this information, we can predict what types of FA we would find in lake trout eggs based on their diet, again following the principle "you are what you eat" and assuming that FA are not transformed and that female fish will transfer all available FA to their progeny.

In our study, we determined that lake trout eggs from Lake Michigan had higher levels of DHA compared to other sample sites in the NL fraction. High levels of DHA indicate alewife consumption by lake trout in Lake Michigan. When we compared the concentrations of 16:1n-7 in the NL fraction, we determined that lake trout eggs from Drummond Island and Grindstone (both Lake Huron) had some of the highest levels of 16:1n-7 while sample sites from Lake Michigan (Milwaukee, Waukegan, Portage Point and Old Mission) had lower levels of 16:1n-7. High levels of 16:1n-7 indicates consumption of round goby by lake trout in Drummond Island and Grindstone. Regardless of location, McKenna *et al*. (unpublished data) found that round goby is increasingly important to lake trout diets in both lakes Michigan and Huron, suggesting that the range expansion of round goby is having a significant impact on the forage base of lake trout. The recent alewife population crash in Lake Huron (Madenjian *et al.* 2006) also provided a window for round goby expansion in Lake Huron and greater inclusion in diet. In terms of FA concentrations, our results provide evidence of round bogy consumption by lake trout in Lake Huron, specifically Drummond Island and Grindstone (assuming that round goby FA concentrations are the same in Lake Huron and Lake Michigan) while round goby consumption

by fish from Lake Michigan is not conclusive. Knowing that alewives are the major prey item in Lake Ontario (Bowlby *et al*. 2007) and Cayuga Lake (Bishop, personal communication 2009), we would expect to find high levels of DHA and low levels of 16:1n-7 in lake trout eggs similar to Lake Michigan sample sites. In our study, we observed that 16:1n-7 concentrations were lower in Hamlin Beach (Ontario) and Taughannock Falls (Cayuga) compared to sample sites from Lakes Michigan and Huron. This indicates that lake trout in Lake Ontario are feeding mostly on alewives and less on round goby compared to fish from Lakes Michigan and Huron (assuming alewife FAS do not differ among lake systems). It is important to note that we know less about lipids and FA in Lake Ontario prey fish (and almost nothing about lipids and FA in Cayuga Lake prey fish) than we do about lipids in FA in prey fish from Lakes Michigan and Huron. However, lipids and FA concentrations of Lake Ontario prey fish are being studied by various research groups; this will help our understanding of the Lake Ontario system. Table 12 summarizes the primary prey items found in the investigated lakes and major fatty acids associated with them. Prey fatty acid data are taken from Czesny *et al.* (2011).

Our nMDS and PCA results help to further illustrate the importance of 16:1n-7 and DHA as these FA contributed largely to the differences in samples sites in the NL fraction. SIMPER routines also showed that 18:1n-9, 16:1n-7, and 18:1n-7 contributed the most to dissimilarity among sample sites in the NL fraction. According to Czseny *et al.* (2011), rainbow smelt (dominant prey in Lake Erie and Lake Champlain) are high in DHA and low in 16:1n-7 compared to other prey from Lake Michigan. In our study, we found that lake trout eggs from Lake Erie had higher levels of 16:1n-7 and low levels of DHA compared to lake trout eggs from Lake Champlain, despite having the same dominant prey item. It is likely that rainbow smelt from these differing lake systems have different FA concentrations, although further

investigation of prey lipids and FA in Lake Erie and Champlain is required. Lastly, lake trout diet in Lake Superior is dominated by lake herring. Unfortunately, little is known about the FAS of lake herring in Lake Superior. Despite this shortcoming, we can attribute differences in FA concentrations in lake trout eggs from Lake Superior to reflect a herring-dominated diet.

Although differences in lipids and FAS were observed in our data, it is difficult to say whether or not these differences are biologically significant. Differences in TL, NL and PL as well as differences in FA require further investigation before the health of the ecosystem can be accurately determined. However, other studies have shown that proper ratios of certain FA can influence fish health and development. For example, Sargent (1995) found that a DHA: EPA ratio of 2:1 is commonly found in PL fraction of fish eggs. Similarly, Czesny *et al*. (2009) found that lake trout had an average DHA: EPA ratio of 3.4 in the PL fraction which is similar to the results found in our study (DHA: EPA range of 3.2-4.7 in the PL fraction). Czesny *et al*. (2009) explained that high levels of DHA and/or low levels of EPA resulted from heavy alewife consumption by adult fish. We know that alewives made up a majority of the diet in lake trout from lakes Michigan, Ontario, and Cayuga Lake thus it is plausible that the ratio of DHA: EPA in this study indicates alewife consumption by lake trout in Lakes Michigan, Ontario, and Cayuga Lake. Other studies have shown AA to increase resistance to infection, lead to better egg quality, and promote growth in salmonids (Ackman and Takeuchi 1986, Bell and Sargent 2003). Sargent (1995) also showed that proper EPA:AA ratio is important to fish health and development. Czesny *et al*. (2009) showed that early mortality syndrome (EMS) increased if the ratio of EPA:AA was below 2 in the NL or below 1 in the PL fraction. In our study we found that the EPA:AA ratio in the NL fraction ranged from 0.4-0.7 and 0.8-1.3 in the PL fraction. Contrary to Czesny *et al.* (2009), we did not find any correlation between EPA:AA ratios and survival.

Similarly, we were not able to determine strong correlations between lipids, fatty acids concentrations, and survival. Other studies have shown relationships between total lipid content of eggs and egg quality in other freshwater fishes. Zhukinsky and Kim (1981) found that high levels of lipids in roach (*Rutilus rutilus*) and bream (*Abramis brama*) eggs increased larval viability whereas Devauchelle *et al.* (1982) found a negative relationship between total lipid content of eggs and egg viability. In a study by Sheikh-Eldin *et al*. (1996), egg viability varied heavily regardless of egg lipid content. Likewise, Czesny and Dabrowski (1998) were unable to demonstrate a definite relationship between total egg lipid content and egg viability in walleye. In the most recent study of lake trout survival, Czesny *et al*. (2012) found the early mortality syndrome (EMS) was positively and negatively correlated to certain fatty acids. For example, 20:1n-9 was positively correlated with EMS mortality while linoleic acid was negatively correlated with EMS mortality. Thus, different concentrations of specific FA may negatively or positively affect embryo survival. Likewise, proper DHA:EPA and EPA:AA ratios (not necessarily high or low concentrations) may also increase embryo survival. Another important factor when considering fish health is the ratio of *n*-3/*n*-6 PUFA. In a study by Leray *et al*. (1985), a low *n*-3/*n*-6 ratio resulted in stunted embryo development, reduction in hatching, and decreased viability in rainbow trout. Likewise Santiago and Reyes (1993) found that the ratio of *n*-3/*n*-6 fatty acids affected the reproductive success of Nile tilapia *Oreochromis nitloticus*. We did not observe any correlations between the ratio of *n*-3/*n*-6 PUFA and survival in either the NL of PL fractions of this study.

Although total lipid content and the concentrations of specific FA are important factors that determine egg quality, they are not the only factors affecting embryo survival. Other factors such as thiamine concentration in lake trout eggs may influence survival of lake trout embryos.

Recently, studies have shown an increase in thiamine levels in lake trout eggs from Lake Michigan and Lake Huron (Riley *et al*. 2011). Low levels of thiamine are associated with EMS in alevins (Honeyfield *et al*. 2005, Czesny *et al*. 2009). Despite increasing levels of thiamine, EMS may still be negatively affecting alevin survival in our study. It is also important to note that sample sizes were very low especially in Hamlin Beach. Larger sample sizes may have been helpful in determining differences between sample sites and correlations with specific FA. Alevins in this study exhibited some signs of EMS including yolk oedema, which is associated with ammonia build up in culture systems (Wolf 1957, Burkahlter and Kaya 1997). Regardless, the relationships among total lipid content, egg quality, and embryo survival remain an area that needs further investigation.

Ideally the best predicative model for predicting percent composition of predator diet incorporates the use of prey FAS of all prey items available within a lake system (and therefore available to predators). Iverson *et al*. (2004) recently proposed the use of Quantitative Fatty Acid Signature Analysis (QFASA) to predict and estimate predator diets. At its core QFASA estimates the proportion of specific prey items in a predator's diet based on analysis of the predators FAS and comparing it known FAS of potential prey items available to that predator. In order to construct this QFASA detailed information of prey FAS must be determined and incorporated into analysis. More recently, Czesny *et al* (2011) publication on the FAS of many prey fish in Lake Michigan has greatly expanded our ability to predict predator diet based on prey FAS. Although this information helped us to determine trends in lake trout egg FAS in this study, a more complete understanding of prey FAS in each lake system (Lake Superior, Lake Erie, Lake Ontario, Cayuga Lake, and Lake Champlain and to a lesser degree Lake Huron) are still required before we can create a model the accurately determines percent composition of predator diet

solely by based on the analysis of predator tissue samples. Even though we know the dominate prey items in each lake system, caution must be taken when predicting percent composition of predator diet based on FAS. Additionally, constantly changing forage bases, most notably the introduction and expansion of round goby and their inclusion in the diet of lake trout, are leading to changes in FAS. Increasing reliance on goby in lake trout diets may have positive implications on lake trout restoration and increase the chances on lake trout rehabilitation in the Great Lakes and Finger Lakes. Continued monitoring of lake trout diets, monitoring of lake trout egg FA concentrations, and better understanding prey FAS will be the utmost importance for the continued recovery of lake trout.

5. Literature Cited

Ackman, R.G., and J. McLachlan. 1977. Fatty acids in some Nova Scotian marine seaweeds: a survey for octadecapentaenoic and other biochemically novel fatty acids. Proceedings of the Nova Scotian Institute of Science 28: 47–64.

Ackman, R.G., and T. Takeuchi. 1986. Comparison of fatty acids and lipids of smolting hatchery-fed and wild Atlantic salmon *Salmo salar*. Lipids 21: 117-120.

Aitchison, J. 1986. The statistical analysis of compositional data. Chapman and Hall, New York.

Almansa, E., M.J. Perez, J.R. Cejas, P. Badia, J.E. Villamandos, and A. Lorenzo. 1999. Influence of broodstock gilthead seabream (*Sparus aurata* L.) dietary fatty acids on egg quality and egg fatty acid composition throughout the spawning season. Aquaculture 170: 323–336.

Bell, J., and J.R. Sargent. 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquaculture 218: 491-499.

Bell, J.G., B.M. Farndale, M.P. Bruce, J.M. Navas, and M. Carillo.1997. Effects of broodstock dietary lipid on fatty acid compositions of eggs from sea bass (*Dicentrarchus labrax*) Aquaculture 149: 107–119.

Bobe, J., and C. Labbé. 2010. Egg and sperm quality in fish. General and Comparative Endocrinology 165: 535-548.

Bowlby, J.N., D. Bishop, M. Daniels, T. Eckert, J. Hoyle, B. Lantry, R. O'Gorman, R. Owens, and T. Schaner. 2007. The offshore pelagic fish community. Pages 75-88 *in* B.J. Morrison and S.R. LaPan, editors. State of Lake Ontario 2003. Great Lakes Fishery Commission Special Publication 07-01.

Bronte, C., C. Krueger, M. Holey, M. Toneys, R. Eshenroder, and J. Jonas. 2008. A guide for the rehabilitation of lake trout in Lake Michigan. Great Lakes Fisheries Commission Miscellaneous Publication 2008-01, Ann Arbor, Michigan.

Budge, S. M., S.J. Iverson, W.D. Bowen, and R.G. Ackman. 2002. Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank and southern Gulf of St. Lawrence. Canadian Journal of Fisheries and Aquatic Sciences 59: 886-898.

Budge, S.M., S.J. Iverson, and H.N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science 59: 886-898.

Burkahlter, D.E., and D.M. Kaya. 1997. Effects of prolonged exposure to ammonia on fertilized eggs and sac fry of rainbow trout (*Salmo gairdneri*). Transactions of the American Fisheries Society 106:470-475.

Czesny, S., and K. Dabrowski. 1998. The effect of egg fatty acid concentrations on embryo viability in wild and domesticated walleye (*Stizostedion vitreum*). Aquatic Living Resources 11: 371-378.

Czesny. S., J. Dettmers, J. Rinchard, and K. Dabrowski. 2009. Linking egg thiamine and fatty acid concentrations of Lake Michigan lake trout with early life stage mortality. Journal of Aquatic Animal Health 21: 262-271.

Czesny, S., J. Rinchard, S.D. Hanson, J.M. Dettmers, and K. Dabrowski. 2011. Fatty acid signatures of Lake Michigan prey fish and invertebrates: among-species differences and spatiotemporal variability. Canadian Journal of Fisheries and Aquatic Sciences 68: 1211-1230.

Czesny, S., J. Rinchard, B. Lee, K. Dabrowski, J.M. Dettmers, and Y. Cao. 2012. Does spatial variation in egg thiamine and fatty concentration of Lake Michigan lake trout *Salvelinus namaycush* lead to differential early mortality syndrome and yolk oedema mortality in offspring. Journal of Fish Biology 80:2475-2493.

Dalsgaard, J., M. John, G. Kattner, D. Muller-Navarra, and W. Hagen. 2003. Fatty acid trophic markers in pelagic marine environment. Advances in Marine Biology 45: 225-340.

Devauchelle, N., G. Brichon, F. Lamour, and G. Stephan. 1982. Biochemical composition of ovules and fecund eggs of sea bass (*Dicentrarchus labrax*), sole (*Solea vulgaris*) and turbot (*Scophthalmus maximus*). Pages 155-157 *in* C.J. Ritcher and H.J. Goos, editors. Reproductive physiology of fish. Center for Agricultural Publishing and Documentation.

Eshmeyer, P. 1955. The reproduction of lake trout in southern Lake Superior. Transactions of the American Fisheries Society 84: 47-74.

Falk-Petersen, S., T. Haug, K.T. Nilssen, A. Wold, and T.M. Dahl. 2004. Lipids and trophic linkages in harp seal (*Phoca groenlandica*) from the eastern Barents Sea. Polar Research 23: 43-50.

Fernandez-Palacios, H., M. Soledad Izquierdo, L. Robaina, A.Valencia, M. Salhi, and J.M. Vergara. 1995. Effect of *n*–3 HUFA level in broodstock diets on egg quality of gilthead sea bream (*Sparus aurata* L.). Aquaculture 132: 325–337.

Fish Stocking. 2012. [Online]. New York State Department of Environmental Conservation, Albany, New York. Available at http://www .dec.ny.gov. Accessed 8 September 2013.

Folch, J., M. Lees, and G.H.S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226: 497-509.

FWS/GLFC 2010. Great Lakes Fish Stocking database. U.S. Fish and Wildlife Service, Region 3 Fisheries Program, and Great Lakes Fishery Commission.

Gorman, O., L. Evrard, G. Cholwek, J. Falck, and M. Vinson. 2010. Status and trends of prey fish populations in Lake Superior, 2009. U.S. Geological Survey, Ashland, Wisconsin USA.

Hile, R., P. Eschmeyer, and G. Lunger. 1951. Decline of the lake trout fishery in Lake Michigan. U.S. Fish and Wildlife Service Fishery Bulletin 52: 77-95.

Honeyfield, D.C., J.P. Hinterkopf, J.D. Fitzsimons, D.E. Tillit, J.L. Zajicek, and S.B. Brown. 2005. Development of thiamine deficiencies and early mortality syndrome in lake trout by feeding experimental and feral fish diets containing thiaminase. Journal of Aquatic Animal Health 17: 4-12.

Iverson, S.J. 1993. Milk secretion in marine mammals in relation to foraging: can milk fatty acids predict diet? Symposia of the Zoological Society of London 66: 263-291.

Iverson, S.J., K.J. Frost, and S.L.C. Lang. 2002. Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound Alaska: factors contributing to among and within species variability. Marine Ecology Progress Series 241: 161-181.

Iverson, S.J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: A new method for estimating predator diets. Ecological Monographs 74: 211-235.

Juaneda, P., and G. Roquelin. 1985. Rapid and convenient separation of phospholipids and nonphosphorus lipids from rat heart using silica cartridges. Lipids 20: 40-41.

Kaitaranta, J.K. 1980. Lipids and fatty acids of whitefish (*Coregonus albula*) flesh and roe. Journal of the Science of Food and Agriculture 31: 1303-1308.

Kaitaranta, J.K., and R.G. Ackman. 1981. Total lipids and lipid classes of fish roe. Comparative Biochemistry Physiology 69B: 725-729.

Kitchell, J.F., S.P. Cox, C.J. Harvey, T.B. Johnson, D.M. Mason, K.K. Schoen, K. Aydin, C. Bronte, M. Ebener, M. Hansen, M. Hoff, S. Schram, D. Schreiner, and C.J. Walters. 2000. Sustainability of the Lake Superior fish community: Interaction in a food web context. Ecosystems 3: 545-560.

Lake Champlain Fisheries Technical Committee. 2009. Strategic Plan for Lake Champlain Fisheries. Lake Champlain Fish and Wildlife Management Cooperative, U.S. Fish and Wildlife Service, Essex Junction, Vermont, USA.

Lake Erie Forage Task Group. 2012. Report of the Lake Erie Forage Task Group, March 2012. Presented to the Standing Technical Committee, Lake Erie Committee of the Great Lakes Fishery commission, Ann Arbor, Michigan, USA.

Leray, C., G. Nonnotte, P. Rouband, and C. Leger. 1985. Incidence of (n-3) essential fatty acid deficiency on trout reproductive processes. Reproduction Nutrition Development 25:567-581.

Lovern, J.A. 1935. Fat metabolism in fishes. The fats of some plankton crustacea. Biochemical Journal 29: 847-849.

Lubzens, E., G. Young, J. Bobe, and J. Cerdà. 2010. Oogensis in teleosts: How fish eggs are formed. General and Comparative Endocrinology 165: 367-389.

Madenjian, C.P., T.J. DeSorcie, and R.M. Stedman. 1998. Ontogenic and special patterns in diet and growth of lake trout in Lake Michigan. Transactions of the American Fisheries Society 127: 236-252.

Madenjian, C.P., J.P. Holuszko, and T.J. Desorcie. 2006. Spring-Summer Diet of Lake Trout on Six Fathom Bank and Yankee Reef in Lake Huron. Journal of Great Lakes Research 32: 200-208.

Madenjian, C.P., D.M. Warner, D.B. Bunnell, R.M. Claramunt, and J.M. Dettmers. 2008. Status of planktivore populations. Pages 49-54 *in* D.F. Clapp and W. Horns, editors. The state of Lake Michigan in 2005. Great Lakes Fishery Commission Special Publication 08-02.

Marsden, J.E., and R.W. Langdon. 2012. The history and future of Lake Champlain's fisheries and fish. Journal of Great Lakes Research 38: 19-34.

Metcalfe, L.D., and A.A. Schmitz. 1961. The rapid preparation of fatty acid ester for gas chromatographic analysis. Analytical Chemistry 33: 363-364.

Miller, M.A., and M.E. Holey. 1992. Diets of lake trout inhabiting nearshore and offshore Lake Michigan environments. Journal of Great Lake Research 18: 51-60.

Napolitano, G.E. 1999. Fatty acids as trophic and chemical markers in freshwater ecosystems. Pages 21-44 *in* M.T. Arts and B.C. Wainman, editors. Lipids in Freshwater Ecology. Springer, New York, New York, USA.

Navas, J.M., M. Bruce, M. Thrush, B.M. Farndale, N. Bromage, S. Zanuy, M. Carrillo, J.G. Bell, and J. Ramos. 1997. The impact of seasonal alteration in the lipid composition of broodstock diets on egg quality in the European sea bass. Journal of Fish Biology 51: 760-773.

Riley, S.C., J.X. He, J.E. Johnson, T.P. O'Brien, and J.S. Schaeffer. 2007. Evidence of widespread natural reproduction in lake trout *Salvelinus namaycush* in the Michigan waters of Lake Huron. Journal of Great Lakes Research 33: 917-921.

Riley, S.C., J. Rinchard, D.C. Honeyfield, A.N. Evans, and L. Begnoche. 2011. Increasing thiamine concentrations in lake trout eggs from lakes Huron and Michigan coincide with low alewife abundance. North American Journal of Fisheries Management 31: 1052-1064.

Rodriguez, C., R.J. Cejas, M.V. Martin, P. Badía, M. Samper, and A. Lorenzo. 1998. Influence of *n*–3 highly unsaturated fatty acid deficiency on the lipid composition of broodstock gilthead seabream (*Sparus aurata* L.) and on egg quality. Fish Physiology and Biochemistry18: 177–187.

Santiago, C.S., and O.S. Reyes. 1993. Effects if dietary lipid sources on reproductive performance and tissue lipid levels on Nile tilapia (*Oreochromis niloticus*) broodstock. Journal of Applied Ichthyology 9: 33-40.

Sargent, J.R. 1995. Origins and functions of egg lipids: nutritional implications. Pages 353-372, in N.R. Bromage and R.J. Roberts. Boodstock management and egg and larval quality. Blackwell Science. Cambridge, MA.

Schaeffer, J.S., and T.P. O'Brien. 2008. Status and trends of pelagic prey fishes in Lake Huron, 2008. U.S. Geological Survey, Ann Arbor, MI, USA.

Sheikh-Eldin, M., S.S. De Silva, T.A. Anderson, and G. Gooley. 1996. Comparison of fatty acid composition of muscle, liver, mature oocytes and diets of wild and captive Macquarie perch, *Macquaria australasica*, broodfish. Aquaculture 144: 201-216.

Smith, S.J., S.J. Iverson, and W.D. Bowen. 1997 Fatty acid signatures of and classification trees: mew tools for investigating the foraging ecology of seals. Canadian Journal of Fisheries and Aquatic Sciences 54: 1377-1386.

Tocher, D.R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science 11: 107-184.

Wiegand, M.D. 1996. Composition, accumulation and utilization of yolk lipids in teleost fish. Reviews of Fish Biology 6: 259–286.

Wiegand, M.D., T.A. Johnston, J. Martin, and W.C. Leggett. 2004. Variation in neutral and polar lipid compositions of ova in ten reproductively isolated populations of walleye (*Sander vitreus*). Canadian Journal of Fisheries and Aquatic Sciences 61: 110-121.

Wolf, K. 1957. Experimental induction of blue-sac disease. Transactions of the American Fisheries Society 86: 61-70.

Zhukinsky, V.N., and Y.D. Kim. 1981. Characteristics of age-related variability in the composition of amino acids and lipids in mature and over ripe eggs of Azov roach (*Rutilus rutilus*) and the bream (*Abramis brama*). Journal of Ichthyology 20: 121-132.

Table 1. Summary of samples including locations (lake and sample site within the lake), sample sizes (*n*) and morphological data (length and weight), and collection agencies for all sites sampled in 2009 and 2010 (- indicates absence of data).

		Sample Site														
		Cayuga Lake	L. Ontario	L. Michigan						L. Huron				L. Champlain	L. Erie	L. Superior
Year		Taughannock Falls[a,b]	Hamlin Beach[b]	Clay Banks[c]	Milwaukee[d]	Waukegan[e,f]	Michigan City[g]	Portage Point[h]	Old Mission[i]	Drummond Island[j]	Grindstone[k]	Owen Sound[l]	Parry Sound[l]	Grand Isle[n]	Hamburg[a]	Klondike Reef[m]
2009	*n*	24	12	21	30	14	14	28	16	25	25	23	5	-	-	9
	Length (cm)	-	73.9±4.5	73.2± 4.4	72.8±3.3	75.5±6.3	76.4±4.0	72.6±5.1	67.6±5.3	67.4±3.4	72.3±5.2	70.6±3.9	73.8±7.1	-	-	47.6±5.9
	Weight (kg)	-	4.3±0.9	-	-	4.3±1.4	4.4±0.8	4.1±1.0	3.1±0.7	3.1±0.6	3.5±1.0	4.0±0.7	3.8±1.2	-	-	0.9±0.3
2010	*n*	26	27	18	18	20	30	26	30	32	-	-	14	20	11	-
	Length (cm)	-	76.8±4.9	75.2±4.9	72.4±5.6	70.9±4.2	76.3±4.4	66.6±13.4	69.8±5.7	71.7±4.6	-	-	71.9±6.2	70.5±5.9	77.6±4.4	-
	Weight (kg)	-	-	4.2±0.9	-	3.5±0.7	4.7±1.1	3.4±0.8	3.4±0.8	3.8±0.9	-	-	3.5±1.1	-	-	-

Collection agencies: a: New York State Department of Environmental Conservation, b: The College at Brockport Department of Environmental Science and Biology, c: US Fish and Wildlife Service, d:Wisconsin Department of Natural Resources, e: Illinois Department of Natural Resources, f: Illinois Natural History Survey, g: Indiana Department of Natural Resources, h: Little River Band of Ottawa Indians, i: Grand Traverse Bay of Ottawa and Chippewa Indians, j: Inter-Tribal Fisheries, k: Michigan Department of Natural Resources, l: Ontario Ministry of Natural Resources, m: La Crosse Fish Health Center, n: Vermont Department of Fish and Wildlife.

Table 2. Pearson's coefficient (r) between total fish length and concentration of individual fatty acids in both the neutral lipid (NL) and phospholipid (PL) fractions of lake trout eggs. Sample size (n) = 435. Significant correlations denoted by an asterisk.

Fatty Acid	Correlations	
SAFA	NL	PL
14:0	0.088	-0.125*
15:0	-0.005	-0.100*
16:0	0.083	-0.002
17:0	0.061	-0.141*
18:0	0.086	0.123*
ΣSAFA	0.116*	0.036
MUFA		
16:1n-9	0.284*	0.156*
16:1n-7	0.164*	0.107*
17:1	-0.036	0.059
18:1n-9	0.108*	0.082
18:1n-7	0.245*	0.309*
20:1	0.066	0.047
ΣMUFA	0.201*	0.274*
PUFA		
18:2n-6	-0.186*	-0.233*
20:2n-6	-0.195*	-0.202*
20:3n-6	-0.013	0.049
20:4n-6	0.033	0.099*
22:4n-6	0.041	0.165*
22:5n-6	-0.141*	-0.100*
Σn6	-0.122*	-0.022
18:3n-3	-0.069	-0.029
18:4n-3	-0.133*	-0.126*
20:3n-3	-0.109*	-0.118*
20:4n-3	-0.191*	-0.172*
20:5n-3	-0.209*	-0.105*
22:5n-3	0.022	0.337*
22:6n-3	-0.273*	-0.317*
Σn3	-0.239*	-0.227*
ΣPUFA	-0.217*	-0.198*
DHAEPA	-0.054	-0.096*
EPAAA	-0.253*	-0.146*

Table 3. Total lipid, neutral lipid, and phospholipid (% of wet weight, mean ± standard deviation) of lake trout eggs from all sample sites. Data were combined for 2009 and 2010. Means with different superscript letters indicated statistical difference ($p < 0.05$). Note that "-" indicates missing data.

	Sample Sites														
	Cayuga L.	L. Ontario	L. Michigan						L. Huron				L. Champlain	L. Erie	L. Superior
	Taughannock Falls	Hamlin Beach	Clay Banks	Milwaukee	Waukegan	Michigan City	Portage Point	Old Mission	Drummond Island	Grindstone	Owen Sound	Parry Sound	Grand Isle	Hamburg	Klondike Reef
2009															
n	24	12	21	30	14	14	28	16	25	25	23	5	-	-	9
TL (%)	9.5±0.7	9.4±0.7	9.7±0.5	9.3±0.3	9.0±0.8	9.3±0.8	8.7±1.0	9.4±0.9	8.6±0.7	8.5±0.6	8.2±0.6	7.9±0.6	-	-	8.7±0.6
NL (% of TL)	53.1±2.3	54.4±1.5	54.3±2.5	55.8±1.7	53.3±3.0	56.0±1.6	55.6±3.4	53.9±1.8	54.3±2.6	53.7±2.2	53.4±1.3	54.4±2.1	-	-	50.7±2.7
PL (% of TL)	46.9±2.3	45.6±1.5	45.7±2.5	44.2±1.7	46.7±3.0	44.0±1.6	44.4±3.4	46.1±1.8	45.7±2.6	46.3±2.2	46.6±1.3	45.6±2.1	-	-	49.3±2.7
2010															
n	26	27	18	18	20	30	26	30	32	-	-	14	20	11	-
TL (%)	7.9±0.9	9.1±0.6	9.3±0.5	9.3±0.5	9.0±0.4	9.2±1.0	8.8±0.6	9.3±0.9	8.9±1.2	-	-	8.0±0.9	8.0±0.6	9.3±0.6	-
NL (% of TL)	53.9±1.6	54.1±6.1	55.8±1.9	54.8±2.6	55.9±1.8	55.1±2.9	54.6±3.7	54.9±2.1	55.4±2.3	-	-	55.8±2.9	55.2±3.2	56.7±2.4	-
PL (% of TL)	46.1±1.6	45.9±6.1	44.2±1.9	45.2±2.6	44.1±1.8	44.9±2.9	45.4±3.7	45.1±2.1	44.6±2.3	-	-	44.2±2.9	44.8±3.2	43.3±2.4	-
2009 and 2010															
n	50	39	39	48	34	44	54	46	57	25	23	19	20	11	9
TL (%)	8.7 ± 1.1^{bcde}	9.2 ± 0.6^{abc}	9.5 ± 0.5^{ab}	9.3 ± 0.4^{ab}	9.0 ± 0.6^{abc}	9.2 ± 2.6^{ab}	8.8 ± 0.9^{bcd}	9.2 ± 0.9^{abc}	8.8 ± 1.0^{bcd}	8.5 ± 0.6^{cde}	8.2 ± 0.6^{de}	8.0 ± 0.8^{e}	8.0 ± 0.6^{de}	9.3 ± 0.6^{ab}	8.7 ± 0.6^{bcde}
NL (%)	53.5 ± 2.0^{b}	54.2 ± 5.1^{ab}	55.0 ± 2.3^{ab}	55.4 ± 2.1^{ab}	54.8 ± 2.6^{ab}	55.4 ± 2.6^{ab}	55.1 ± 3.6^{ab}	54.6 ± 2.0^{ab}	54.9 ± 2.5^{ab}	53.7 ± 2.2^{b}	53.4 ± 1.3^{b}	55.4 ± 2.7^{ab}	55.2 ± 3.2^{ab}	56.7 ± 2.4^{a}	50.7 ± 2.7^{c}
PL (%)	46.5 ± 2.0^{b}	45.8 ± 5.1^{bc}	45.0 ± 2.3^{bc}	44.6 ± 2.2^{bc}	45.2 ± 2.6^{bc}	44.6 ± 2.6^{bc}	44.9 ± 3.6^{bc}	45.4 ± 2.0^{bc}	45.1 ± 2.5^{bc}	46.3 ± 2.2^{b}	46.6 ± 1.3^{b}	44.6 ± 2.7^{bc}	44.8 ± 3.2^{bc}	43.3 ± 2.4^{c}	49.3 ± 2.7^{a}

Table 4. Percent detected fatty acids in the neutral lipid fraction of lake trout eggs from all sample sites. Means with different superscript letters indicated statistical difference ($p < 0.05$).

Fatty Acid	Sample Site														
	Cayuga L.	L. Ontario	L. Michigan						L. Huron				L. Champlain	L. Erie	L. Superior
	Taughannock Falls	Hamlin Beach	Clay Banks	Milwaukee	Waukegan	Michigan City	Portage Point	Old Mission	Drummond Island	Grindstone	Owen Sound	Parry Sound	Grand Isle	Hamburg	Klondike Reef
SAFA															
14:0	3.1±0.4^{a}	2.6±0.2^{bc}	2.1±0.3^{de}	2.2±0.3^{de}	2.1±0.2^{de}	2.4±0.0^{bcd}	2.3±0.4^{cde}	2.2±0.2^{de}	2.3±0.4^{cde}	2.0±0.2^{e}	2.2±0.2^{de}	2.2±0.3^{de}	2.3±0.3^{cde}	2.8±0.2^{ab}	2.3±0.3^{cde}
15:0	0.6±0.1^{a}	0.4±0.0^{b}	0.2±0.1^{ef}	0.2±0.0^{def}	0.3±0.0^{cde}	0.3±0.0^{cd}	0.3±0.1^{cde}	0.3±0.0^{de}	0.3±0.0^{cde}	0.3±0.0^{cd}	0.2±0.0^{f}	0.3±0.1^{cd}	0.3±0.0^{cd}	0.3±0.0^{bc}	0.3±0.1^{cd}
16:0	10.3±0.9^{abcde}	10.1±0.7^{abcde}	10.1±1.0^{abcde}	9.9±0.9^{bcde}	9.4±1.0^{de}	9.8±1.0^{bcde}	9.9±1.3^{bcde}	10.1±0.8^{abcde}	9.8±0.7^{bcde}	11.1±0.8^{a}	9.3±0.7^{e}	10.5±1.2^{a}	10.8±0.7^{ab}	10.6±0.7^{abc}	9.6±1.0^{cde}
17:0	0.3±0.0^{a}	0.2±0.0^{b}	0.3±0.2^{a}	0.1±0.0^{bc}	0.3±0.2^{a}	0.1±0.0^{bc}	0.1±0.0^{bc}	0.1±0.0^{bc}	0.1±0.0^{c}	0.1±0.0^{bc}	0.1±0.0^{bc}	0.1±0.0^{bc}	0.2±0.0^{bc}	0.1±0.0^{bc}	0.2±0.0^{bc}
18:0	1.9±0.3^{bcd}	1.6±0.1^{def}	1.9±0.3^{abc}	2.0±0.2^{ab}	1.9±0.3^{bcd}	1.8±0.3^{bcde}	1.8±0.3^{bcde}	1.7±0.2^{bcde}	1.5±0.2^{ef}	1.8±0.2^{bcd}	1.6±0.2^{def}	2.2±0.2^{a}	1.8±0.2^{bcd}	1.7±0.2^{cdef}	1.5±0.2^{f}
ΣSAFA	16.2±1.4^{a}	14.8±0.9^{bcd}	14.6±1.2^{bcde}	14.4±1.3^{bcde}	13.9±1.3^{cde}	14.4±1.3^{bcde}	14.5±1.6^{bcde}	14.4±1.0^{bcde}	13.9±0.9^{cde}	15.4±1.1^{ab}	13.3±3.3^{e}	15.2±1.4^{abc}	15.5±1.0^{ab}	15.5±0.7^{ab}	13.8±1.4^{de}
MUFA															
16:1n-9	1.5±0.2^{abcd}	1.6±0.3^{ab}	1.2±0.3^{ef}	1.1±0.2^{ef}	1.2±0.3^{cdef}	1.3±0.2^{bcdef}	1.2±0.2^{def}	1.4±0.2^{bcde}	1.3±0.3^{cdef}	1.5±0.2^{abc}	1.5±0.3^{abc}	1.8±0.3^{a}	1.8±0.3^{a}	1.5±0.1^{abc}	1.1±0.2^{f}
16:1n-7	7.4±0.8^{f}	7.9±0.8^{ef}	9.7±1.5^{bcde}	9.1±1.4^{cdef}	9.1±1.8^{cdef}	9.9±2.0^{bcd}	8.5±1.9^{def}	8.8±2.3^{cdef}	10.6±1.3^{abc}	11.2±1.8^{ab}	9.5±1.7^{bcde}	8.9±2.0^{cdef}	8.0±0.8^{def}	12.3±1.1^{a}	9.3±0.8^{cdef}
17:1	0.8±0.1^{a}	0.6±0.1^{bc}	0.3±0.2^{b}	0.4±0.0^{fg}	0.2±0.2^{h}	0.4±0.0^{efg}	0.4±0.1^{defg}	0.4±0.1^{defg}	0.4±0.0^{g}	0.5±0.1^{bcd}	0.5±0.1^{cdef}	0.5±0.1^{bcde}	0.6±0.0^{bc}	0.6±0.1^{b}	0.4±0.0^{efg}
18:1n-9	25.0±2.0^{cde}	24.0±1.4^{de}	23.3±2.1^{de}	23.6±1.7^{de}	23.1±2.1^{de}	23.7±2.3^{de}	21.8±2.1^{e}	25.1±2.4^{cde}	24.6±2.7^{de}	26.7±3.0^{abc}	27.6±1.7^{ab}	29.0±3.1^{a}	28.6±2.0^{a}	25.0±1.3^{cde}	24.2±3.8^{def}
18:1n-7	4.8±0.5^{e}	5.1±0.4^{de}	6.5±0.9^{bc}	6.7±0.8^{abc}	6.7±1.1^{abc}	6.7±1.0^{abc}	6.2±0.9^{bc}	5.9±1.1^{cd}	6.2±0.6^{bc}	7.5±1.2^{a}	6.9±0.6^{ab}	6.4±1.2^{bc}	4.9±0.5^{de}	7.2±0.5^{ab}	6.3±0.6^{bc}
20:1	1.3±0.2^{ab}	1.2±0.2^{abcd}	1.1±0.2^{abcd}	1.2±0.2^{ab}	1.2±0.2^{abcd}	1.2±0.3^{abc}	1.0±0.2^{bcd}	1.2±0.3^{ab}	0.9±0.2^{d}	1.0±0.3^{cd}	1.2±0.2^{abc}	1.2±0.2^{abc}	1.2±0.1^{abcd}	1.0±0.2^{bcd}	1.3±0.2^{a}
ΣMUFA	40.8±2.5^{def}	40.3±2.2^{ef}	42.0±4.1^{def}	42.2±3.6^{def}	41.5±4.8^{def}	43.3±4.7^{bcdef}	39.2±4.2^{f}	42.9±5.6^{cdef}	44.0±3.5^{bcde}	48.5±5.0^{a}	47.2±3.3^{abc}	47.7±5.7^{ab}	45.1±2.7^{abcd}	47.6±2.0^{ab}	42.5±4.^{6defdef}
PUFA															
18:2n-6	4.0±1.0^{abc}	4.1±0.3^{abc}	3.5±0.5^{bcde}	3.6±0.4^{bcde}	3.8±0.6^{abcd}	3.7±0.7^{abcde}	3.9±0.6^{abcd}	3.8±0.5^{abcd}	4.1±0.5^{abc}	3.5±0.9^{bcde}	3.0±0.7^{e}	3.3±0.8^{cde}	3.6±0.3^{bcde}	3.2±0.3^{de}	4.4±0.5^{a}
20:2n-6	0.5±0.1^{cd}	0.7±0.1^{b}	0.7±0.1^{b}	0.7±0.1^{b}	0.7±0.1^{b}	0.7±0.2^{b}	0.7±0.2^{b}	0.6±0.2^{bc}	0.6±0.1^{b}	0.4±0.1^{d}	0.4±0.1^{d}	0.4±0.1^{d}	0.4±0.0^{d}	0.3±0.1^{d}	1.0±0.1^{a}
20:3n-6	0.5±0.1^{a}	0.4±0.0^{bcd}	0.4±0.1^{bcde}	0.4±0.1^{abc}	0.4±0.1^{ab}	0.4±0.1^{ab}	0.4±0.1^{ab}	0.3±0.1^{cdef}	0.3±0.0^{ef}	0.3±0.1^{ef}	0.3±0.1^{def}	0.3±0.0^{fg}	0.3±0.0^{cdef}	0.2±0.0^{g}	0.4±0.0^{ab}
20:4n-6	4.9±0.6^{a}	4.6±0.4^{abc}	4.5±0.5^{abc}	4.6±0.5^{abc}	4.5±0.7^{abc}	4.7±0.5^{ab}	4.8±0.6^{a}	4.4±0.5^{abcd}	4.1±0.4^{bcd}	4.1±0.4^{cd}	4.6±0.5^{abc}	3.8±0.6^{de}	3.3±0.4^{ef}	3.3±0.3^{ef}	3.1±0.3^{f}
22:4n-6	0.7±0.2^{ab}	0.6±0.1^{abc}	0.8±0.3^{a}	0.6±0.1^{ab}	0.6±0.1^{ab}	0.6±0.1^{ab}	0.7±0.1^{ab}	0.6±0.1^{bcd}	0.5±0.1^{bcd}	0.5±0.1^{cde}	0.6±0.1^{bcd}	0.4±0.1^{de}	0.4±0.0^{e}	0.4±0.0^{e}	0.4±0.1^{de}
22:5n-6	1.0±0.2^{abcd}	0.8±0.2^{bcde}	0.8±0.5^{bcde}	1.1±0.2^{ab}	1.1±0.3^{abc}	1.0±0.3^{abcd}	1.3±0.3^{a}	1.2±0.2^{a}	0.9±0.3^{bcde}	0.6±0.2^{e}	0.8±0.2^{bcde}	0.7±0.2^{de}	0.6±0.1^{e}	0.7±0.1^{de}	0.8±0.2^{cde}
Σ*n*-6	11.5±0.9^{a}	11.3±0.6^{ab}	10.6±1.1^{abcd}	11.0±1.0^{ab}	11.2±1.5^{ab}	11.2±1.3^{ab}	11.8±1.3^{a}	10.9±1.2^{abc}	10.5±0.8^{abcd}	9.4±1.2^{def}	9.7±1.3^{cdef}	9.0±1.5^{efg}	8.6±0.7^{fg}	8.1±0.6^{g}	10.2±0.8^{bcde}

18:3n-3	4.4±0.7^{abc}	4.5±0.4^{ab}	3.2±0.5^{def}	3.2±0.6^{def}	3.4±0.7^{de}	3.1±0.7^{defg}	3.6±0.7^{cde}	3.2±0.9^{def}	2.9±0.5^{efg}	2.3±0.8^{g}	2.3±0.6^{g}	2.5±1.3^{fg}	5.0±0.4^{a}	3.7±0.6^{bcd}	3.3±0.6^{def}
18:4n-3	1.0±0.2^{b}	0.9±0.2^{b}	0.6±0.1^{cdef}	0.7±0.2^{bcde}	0.7±0.2^{bcde}	0.7±0.2^{bcde}	0.8±0.2^{bcd}	0.6±0.2^{def}	0.6±0.2^{cdef}	0.5±0.2^{f}	0.5±0.1^{f}	0.5±0.3^{ef}	1.3±0.3^{a}	0.8±0.2^{bcd}	0.9±0.3^{bc}
20:3n-3	0.6±0.1^{defg}	1.2±0.2^{a}	0.9±0.2^{bc}	0.9±0.2^{bc}	0.9±0.2^{bc}	0.8±0.2^{bcd}	1.0±0.3^{ab}	0.8±0.3^{bcd}	0.8±0.2^{bcd}	0.4±0.2^{fg}	0.4±0.1^{g}	0.5±0.3^{efg}	0.7±0.1^{cdef}	0.4±0.1^{fg}	1.0±0.1^{ab}
20:4n-3	2.3±0.4^{bcde}	3.2±0.4^{a}	2.7±0.7^{abc}	2.6±0.7^{abc}	2.9±0.9^{abc}	2.4±0.8^{abcd}	3.1±0.8^{ab}	2.6±1.1^{abc}	2.8±0.8^{abc}	1.4±0.9^{e}	2.1±0.6^{cde}	1.7±0.9^{de}	2.8±0.3^{abc}	1.6±0.3^{de}	3.1±0.5^{ab}
20:5n-3	6.6±0.8^{bc}	7.3±0.7^{ab}	6.9±1.0^{abc}	6.9±0.9^{abc}	7.0±0.7^{abc}	7.3±1.2^{ab}	7.1±1.0^{abc}	6.9±1.0^{abc}	7.2±0.7^{abc}	6.5±1.1^{bc}	7.4±0.7^{ab}	6.2±1.0^{c}	6.6±0.5^{bc}	7.0±0.6^{abc}	7.9±1.4^{a}
22:5n-3	4.6±0.7^{cde}	5.2±0.3^{ab}	5.0±0.4^{abc}	5.1±0.5^{abc}	5.3±0.5^{a}	5.1±0.5^{abc}	5.2±0.6^{ab}	4.7±0.5^{abcd}	4.9±0.5^{abcd}	4.7±0.7^{bcd}	4.8±0.4^{abcd}	4.3±0.5^{de}	3.9±0.4^{e}	4.6±0.3^{cde}	4.9±0.6^{abcd}
22:6n-3	12.0±1.3^{abcde}	11.2±1.3^{bcde}	12.9±1.6^{ab}	13.0±1.4^{ab}	12.4±1.6^{abcd}	11.7±1.7^{bcde}	13.6±1.9^{a}	12.9±1.8^{ab}	12.3±1.2^{abcde}	10.9±1.5^{cde}	12.2±1.2^{abcde}	12.2±1.5^{abcde}	10.5±1.5^{cd}	10.6±0.6^{de}	12.5^{abc}±1.2
ΣPUFA	43.0±2.6^{ab}	44.9±2.5^{a}	42.8±4.4^{ab}	43.4±4.0^{ab}	43.8±4.9^{ab}	42.3±4.9^{ab}	46.3±5.0^{a}	42.7±5.9^{ab}	42.1±3.4^{ab}	36.1±5.2^{c}	39.5±3.5^{bc}	37.1±5.9^{c}	39.5±3.0^{bc}	36.9±2.1^{c}	43.7±3.^{ab}8
Σ*n*-3	31.5±2.5^{abcd}	33.6±2.0^{a}	32.2±3.5^{abc}	32.4±3.1^{abc}	32.7±3.6^{ab}	31.1±3.7^{abcd}	34.6±4.0^{a}	31.8±4.8^{abcd}	31.6±2.7^{abcd}	26.7±4.2^{e}	29.8±2.6^{bcde}	28.0±4.7^{de}	30.9±2.4^{abcd}	28.8±1.5^{cde}	33.5±3.1^{ab}
Σn-3/n-6	2.8±0.3^{a}	3.0±0.1^{defg}	3.1±0.2^{cdef}	2.9±0.2^{defg}	2.9±0.2^{defg}	2.8±0.2^{fg}	2.9±0.2^{defg}	2.9±0.2^{defg}	3.0±0.2^{defg}	2.8±0.3^{efg}	3.1±0.5^{cde}	3.1±0.4^{cd}	3.6±0.2^{a}	3.6±0.2^{ab}	3.3±0.2^{bc}
DHA/EPA	1.8±0.4^{abc}	1.5±0.2^{d}	1.9±0.3^{abc}	1.9±0.3^{ab}	1.8±0.2^{abcd}	1.6±0.3^{bcd}	1.9±0.3^{ab}	1.9±0.2^{abc}	1.7±0.2^{abcd}	1.8±0.3^{abcd}	1.7±0.2^{bcd}	2.0±0.3^{a}	1.6±0.2^{cd}	1.5±0.1^{d}	1.6±0.4^{bcd}
ARA/EPA	0.7±0.1^{a}	0.6±0.1^{bc}	0.7±0.1^{abc}	0.7±0.1^{ab}	0.6±0.1^{bc}	0.7±0.1^{abc}	0.7±0.1^{ab}	0.6±0.1^{bc}	0.6±0.1^{cd}	0.6±0.1^{bc}	0.6±0.1^{bc}	0.6±0.1^{bc}	0.5±0.0^{de}	0.5±0.0^{ef}	0.4±0.1^{f}

Table 5. Percent detected fatty acids in the phospholipid fraction of lake trout eggs from all sample sites. Means with different superscript letters indicated statistical difference ($p < 0.05$).

Fatty Acid	Sample Site														
	Cayuga L.	L.Ontario	L. Michigan						L. Huron				L. Champlain	L Erie	L.Superior
	Taughannock Falls	Hamlin Beach	Clay Banks	Milwaukee	Waukegan	Michigan City	Portage Point	Old Mission	Drummond Island	Grindstone	Owen Sound	Parry Sound	Grand Isle	Hamburg	Klondike Reef
SAFA															
14:0	1.1±0.1^{abc}	1.1±0.1^{ab}	1.0±0.1^{cd}	1.0±0.1^{bcd}	1.0±0.1^{bcd}	1.1±0.1^{abc}	1.1±0.1^{abc}	1.1±0.2^{abc}	1.2±0.2^{a}	0.9±0.1^{d}	1.1±0.1^{abc}	1.0±0.2^{bc}	1.2±0.1^{ab}	1.3±0.1^{a}	1.1±0.1^{b}
15:0	0.6±0.1^{a}	0.4±0.0^{b}	0.3±0.0^{de}	0.3±0.0^{e}	0.3±0.0^{de}	0.3±0.1^{cde}	0.3±0.0^{cde}	0.3±0.1^{cde}	0.3±0.0^{cd}	0.3±0.0^{cde}	0.3±0.0^{de}	0.3±0.1^{cde}	0.3±0.0^{cde}	0.4±0.0^{bc}	0.4±.1^{bc}
16:0	16.0±1.2^{abc}	15.5±1.9^{bc}	15.4±0.8^{bc}	16.0±0.9^{abc}	14.6±1.3^{c}	16.1±0.9^{abc}	16.3±1.1^{ab}	17.1±2.3^{a}	16.7±1.5^{ab}	16.8±0.7^{ab}	16.2±0.7^{ab}	17.1±1.0^{a}	17.1±1.1^{a}	17.0±1.1^{ab}	16.3±1.0^{ab}
17:0	0.5±0.1^{a}	0.3±0.0^{b}	0.2±0.1^{de}	0.2±0.0^{de}	0.2±0.0^{e}	0.2±0.0^{de}	0.3±0.0^{cd}	0.3±0.1^{bcd}	0.2±0.0^{de}	0.2±0.1^{de}	0.2±0.0^{de}	0.3±0.1^{cd}	0.3±0.0^{bc}	0.3±0.0^{bcd}	0.3±0.0^{bcd}
18:0	6.9±0.4^{abcd}	6.0±1.0^{de}	6.6±0.6^{cd}	6.8±0.6^{abcd}	6.2±0.8^{cd}	6.4±0.7^{cd}	6.7±0.7^{bcd}	6.8±1.0^{abcd}	6.6±0.8^{cd}	7.0±0.6^{abc}	6.8±0.5^{abcd}	7.6±1.2^{a}	6.7±0.6^{abcd}	7.6±0.8^{ab}	5.3±0.7^{e}
ΣSAFA	25.1±1.2^{abcd}	23.3±2.9^{de}	23.5±1.1^{cde}	24.3±1.2^{bcd}	22.4±1.8^{e}	24.1±1.3^{bcde}	24.7±1.3^{abcd}	25.6±2.8^{ab}	25.0±1.8^{abcd}	25.3±1.0^{abc}	24.6±1.0^{abcd}	26.4±1.7^{a}	25.6±1.2^{ab}	26.4±1.5^{a}	23.4±0.9^{cde}
MUFA															
16:1n-9	1.0±0.2^{bcdefg}	1.0±0.2^{bcd}	0.8±0.2^{g}	0.8±0.1^{g}	0.8±0.2^{efg}	0.9±0.2^{cdefg}	0.8±0.2^{fg}	1.0±0.2^{bcdef}	1.0±0.2^{bcdefg}	1.0±0.2^{bcde}	1.1±0.2^{bc}	1.3±0.2^{a}	1.1±0.2^{ab}	1.0±0.1^{bcdefg}	0.8±0.2^{defg}
16:1n-7	1.5±0.1^{f}	1.8±0.3^{def}	2.0±0.4^{cde}	1.9±0.3^{cde}	2.0±0.4^{cde}	2.2±0.4^{bcd}	1.9±0.3^{cde}	1.9±0.5^{cde}	2.6±0.4^{a}	2.5±0.3^{ab}	2.1±0.3^{bcde}	2.0±0.4^{cde}	1.8±0.2^{ef}	2.6±0.1^{a}	2.3±0.2^{abc}
17:1	0.3±0.0^{a}	0.2±0.0^{bcde}	0.2±0.1^{bc}	0.1±0.0^{f}	0.2±0.1^{b}	0.2±0.0^{bcde}	0.2±0.0^{cdef}	0.2±0.0^{cdef}	0.1±0.0^{f}	0.2±0.0^{cdef}	0.2±0.0^{def}	0.2±0.0^{cdef}	0.2±0.0^{bcde}	0.2±0.0^{bcd}	0.1±0.0^{f}
18:1n-9	10.0±0.6^{abcd}	10.3±0.8^{abcd}	9.8±0.6^{cde}	10.2±0.7^{abcd}	9.7±0.5^{de}	9.9±0.6^{bcde}	10.1±0.8^{abcd}	10.1±1.2^{abcd}	9.6±1.3^{de}	9.9±0.9^{bcde}	11.0±0.8^{a}	10.8±1.0^{ab} c	11.0±0.9^{ab}	10.3±0.7^{abcd}	9.0±0.5^{e}
18:1n-7	4.7±0.5^{f}	5.7±0.6^{de}	6.2±0.8^{bcd}	6.2±0.7^{bcd}	6.5±1.0^{bcd}	6.7±0.9^{abc}	6.1±0.8^{bcde}	5.8±1.2^{cde}	6.1±0.9^{bcd}	6.9±0.7^{ab}	6.9±0.6^{ab}	5.9±0.8^{cde}	5.2±0.5^{ef}	7.6±0.4^{a}	5.8±0.5^{cde}
20:1	2.8±0.4^{a}	2.6±0.5^{ab}	2.4±0.5^{abccde}	2.3±0.3^{abcde}	2.4±0.3^{abcd}	2.2±0.3^{bcdef}	2.0±0.4^{efg}	2.2±0.3^{bcdef}	1.8±0.4^{fg}	1.6±0.4^{g}	2.3±0.5^{abcde}	2.1±0.3^{cdef}	2.5±0.4^{abc}	2.0±0.3^{defg}	2.4±0.5^{abcde}
ΣMUFA	20.2±1.0^{d}	21.6±1.3^{bcd}	21.4±1.3^{bcd}	21.6±1.4^{bcd}	21.6±1.4^{bcd}	22.0±1.5^{abc}	21.0±1.5^{bcd}	21.2±2.3^{bcd}	21.3±2.1^{bcd}	22.1±1.4^{abc}	23.6±1.2^{a}	22.3±1.9^{ab}	21.8±1.4^{bcd}	23.6±1.1^{a}	20.4±1.3^{cd}
PUFA															
18:2n-6	1.0±0.3^{ab}	1.0±0.1^{abc}	0.9±0.1^{abcdef}	0.8±0.1^{bcdefg}	0.9±0.1^{abcde}	0.8±0.2^{bcdef}	0.9±0.2^{abcde}	0.8±0.1^{bcdef}	0.9±0.1^{abcd}	0.7±0.2^{defg}	0.6±0.1^{g}	0.7±0.1^{fg}	0.8±0.1^{cdefg}	0.7±0.1^{efg}	1.0±0.1^{a}
20:2n-6	0.8±0.2^{cde}	1.2±0.2^{ab}	1.2±0.3^{ab}	0.9±0.2^{bcd}	1.2±0.3^{ab}	1.0±0.3^{bc}	1.0±0.3^{bc}	0.9±0.3^{bcd}	1.1±0.3^{b}	0.6±0.2^{e}	0.7±0.1^{de}	0.6±0.3^{e}	0.6±0.1^{e}	0.5±0.1^{e}	1.4±0.2^{a}
20:3n-6	0.2±0.1^{ab}	0.1±0.0^{cd}	0.2±0.0^{c}	0.2±0.0^{bc}	0.2±0.1^{a}	0.2±0.0^{cd}	0.2±0.0^{c}	0.1±0.0^{de}	0.1±0.0^{de}	0.1±0.0^{de}	0.1±0.0^{de}	0.1±0.0^{de}	0.1±0.0^{cd}	0.1±0.0^{e}	0.1±0.0^{cd}
20:4n-6	9.5±0.9^{a}	8.5±0.5^{bcd}	8.2±0.8^{bcde}	8.6±0.6^{abc}	9.0±0.7^{ab}	8.5±0.8^{bcd}	8.4±0.7^{bcd}	8.0±0.8^{cdef}	7.2±0.6^{fg}	8.1±0.9^{cde}	7.5±0.6^{efg}	7.9±0.9^{cdef}	7.6±0.6^{defg}	6.9±0.4^{g}	6.8±0.3^{g}
22:4n-6	0.5±0.1^{a}	0.4±0.1^{abcde}	0.5±0.1^{ab}	0.4±0.1^{abcd}	0.5±0.1^{abc}	0.4±0.1^{abcd}	0.4±0.1^{bcdef}	0.4±0.1^{cdef}	0.4±0.1^{cdef}	0.4±0.1^{cdef}	0.4±0.1^{cdef}	0.3±0.1^{ef}	0.3±0.0^{f}	0.3±0.0^{def}	0.3±0.1^{f}
22:5n-6	1.6±0.3^{ab}	1.2±0.2^{bcd}	1.7±0.4^{a}	1.7±0.3^{a}	1.7±0.5^{a}	1.5±0.5^{abc}	1.7±0.4^{a}	1.5±0.3^{abc}	1.3±0.3^{bcd}	1.0±0.3^{d}	1.1±0.2^{d}	1.0±0.3^{d}	1.0±0.2^{d}	1.0±0.2^{d}	1.2±0.2^{cd}
Σ*n*-6	13.6±1.1^{a}	12.4±0.9^{ab}	12.6±1.5^{ab}	12.7±0.8^{ab}	13.5±1.3^{a}	12.4±1.4^{ab}	12.6±1.1^{ab}	11.8±1.2^{bc}	11.0±1.1^{cd}	10.9±1.0^{cd}	10.3±0.8^{de}	10.6±1.1^{cd} e	10.5±0.8^{de}	9.6±0.7^{e}	10.9±0.8^{cd}
18:3n-3	0.7±0.1^{ab}	0.7±0.1^{a}	0.4±0.1^{cde}	0.4±0.1^{cdef}	0.5±0.1^{c}	0.4±0.1^{cde}	0.5±0.1^{cd}	0.4±0.2^{cde}	0.5±0.1^{cd}	0.3±0.1^{ef}	0.3±0.1^{f}	0.3±0.2^{def}	0.7±0.1^{a}	0.5±0.1^{cd}	0.5±0.1^{cd}

18:4n-3	0.1±0.0^{ab}	0.1±0.0^{ab}	0.1±0.1^{bc}	0.1±0.0^{bc}	0.1±0.0^{ab}	0.1±0.0^{bc}	0.1±0.0^{bc}	0.1±0.1^{bc}	0.1±0.0^{bc}	0.0±0.0^{c}	0.0±0.0^{c}	0.0±0.0^{c}	0.1±0.0^{ab}	0.1±0.0^{bc}	0.1±0.0^{bc}
20:3n-3	0.6±0.1^{defg}	1.2±0.2^{a}	1.0±0.3^{ab}	0.7±0.2^{bcd}	0.9±0.3^{bc}	0.7±0.3^{bcde}	0.9±0.2^{ab}	0.7±0.3^{bcd}	0.8±0.3^{bcd}	0.3±0.2^{g}	0.4±0.1^{fg}	0.4±0.3^{efg}	0.6±0.1^{cdef}	0.4±0.1^{fg}	0.7±0.2^{bcd}
20:4n-3	0.6±0.1^{abc}	0.8±0.1^{a}	0.7±0.2^{ab}	0.6±0.1^{abc}	0.7±0.2^{ab}	0.6±0.2^{abcd}	0.7±0.2^{ab}	0.5±0.2^{bcde}	0.7±0.2^{ab}	0.3±0.2^{f}	0.4±0.1^{cdef}	0.4±0.2^{def}	0.7±0.1^{ab}	0.3±0.1^{ef}	0.7±0.1^{ab}
20:5n-3	7.5±0.8^{bcd}	7.5±0.5^{bc}	6.7±0.6^{def}	6.9±0.5^{cdef}	7.3±0.5^{bcde}	7.2±0.8^{bcde}	6.6±0.7^{ef}	6.5±0.6^{ef}	6.5±0.7^{ef}	6.7±0.9^{cdef}	6.2±0.8^{f}	6.2±0.7^{f}	8.0±0.6^{ab}	7.8±0.7^{b}	8.8±1.2^{a}
22:5n-3	5.0±0.9^{cd}	6.2±0.7^{ab}	5.6±1.2^{abcd}	5.0±0.8^{cd}	5.8±1.4^{abcd}	5.9±1.2^{abcd}	4.8±0.9^{d}	5.0±1.0^{cd}	5.5±0.7^{abcd}	6.6±1.2^{a}	5.3±0.6^{bcd}	5.1±0.7^{bcd}	5.2±0.4^{bcd}	6.1±0.7^{abc}	4.9±0.2^{d}
22:6n-3	26.7±1.5^{bcd}	26.3±1.8^{cd}	28.2±1.9^{abc}	27.8±1.6^{abc}	27.3±1.7^{bcd}	26.6±1.4^{bcd}	28.2±2.1^{abc}	28.1±2.7^{abc}	28.7±2.1^{ab}	27.4±2.4^{abcd}	28.8±1.6^{ab}	28.3±2.5^{ab} c	26.7±1.6^{bcd}	25.2±1.4^{d}	29.6±2.0^{a}
Σ*n*-3	41.1±1.2^{bc}	42.7±2.0^{b}	42.5±1.8^{bc}	41.4±1.7^{bc}	42.5±1.6^{bc}	41.4±1.5^{bc}	41.7±1.7^{bc}	41.4±3.5^{bc}	42.8±2.5^{b}	41.7±1.7^{bc}	41.5±1.5^{bc}	40.8±2.3^{bc}	42.1±1.5^{bc}	40.4±1.1^{c}	45.3±1.0^{a}
ΣPUFA	54.7±1.2^{abc}	55.1±2.6^{ab}	55.1±1.7^{ab}	54.1±1.7^{abcd}	56.0±2.1^{a}	53.9±1.8^{abcde}	54.3±1.7^{abc}	53.2±3.9^{bcde}	53.7±2.9^{abcde}	52.6±1.5^{cde}	51.8±1.4^{def}	51.4±2.4^{ef}	52.6±1.7^{cde}	50.0±0.9^{f}	56.2±1.2^{a}
Σn-3/n-6	3.0±0.3^{e}	3.4±0.2^{cde}	3.4±0.6^{cde}	3.3±0.3^{de}	3.2±0.3^{de}	3.4±0.4^{de}	3.3±0.4^{de}	3.5±0.4^{bcd}	3.9±0.4^{ab}	3.9±0.5^{abc}	4.1±0.4^{a}	3.9±0.5^{ab}	4.0±0.4^{a}	4.2±0.4^{a}	4.2±0.4^{a}
DHA/EPA	3.6±0.6^{cde}	3.5±0.3^{de}	4.3±0.6^{ab}	4.1±0.4^{abcd}	3.8±0.3^{bcde}	3.7±0.5^{bcde}	4.3±0.6^{ab}	4.3±0.5^{ab}	4.5±0.6^{a}	4.2±0.8^{abc}	4.7±0.6^{a}	4.6±0.9^{a}	3.3±0.3^{e}	3.2±0.4^{e}	3.5±0.7^{de}
ARA/EPA	1.3±0.1^{a}	1.1±0.1^{bc}	1.3±0.2^{abc}	1.3±0.1^{abc}	1.2±0.1^{abc}	1.2±0.2^{abc}	1.3±0.1^{a}	1.2±0.1^{abc}	1.1±0.1^{c}	1.2±0.2^{abc}	1.2±0.2^{abc}	1.3±0.1^{ab}	1.0±0.1^{d}	0.9±0.1^{de}	0.8±0.1^{e}

Table 6. ANOSIM of fatty acid signatures among lake trout eggs across all sample sites in the neutral lipid fraction of lipids. Values are R-statistics, which range between 0.0 and 1.0, with a value of 1.0 indicating that "within-group" samples are more similar to one another than they are to samples in another group, and a value of 0.0 indicating that the "within-group" samples are no more similar to one another than they are to samples in another group. "*" indicates a differences in FAS between sites.

Sample site	Taughannock Falls	Hamlin Beach	Clay Banks	Klondike Reef	Drummond Island	Owen Sound	Portage Point	Waukegan	Old Mission	Michigan City	Grindstone	Milwaukee	Parry Sound	Grand Isle	Hamburg
Taughannock Falls	-	0.391*	0.579*	0.725*	0.683*	0.681*	0.495*	0.543*	0.373*	0.471*	0.734*	0.55*	0.619*	0.554*	0.715*
Hamlin Beach		-	0.521*	0.798*	0.554*	0.856*	0.338*	0.462*	0.290*	0.344*	0.790*	0.543*	0.757*	0.791*	0.916*
Clay Banks			-	0.289*	0.219*	0.400*	0.118*	0.053	0.143*	0.079	0.405*	0.081	0.523*	0.744*	0.424*
Klondike Reef				-	0.322	0.648*	0.258*	0.200	0.240	0.197	0.444*	0.468*	0.391*	0.867*	0.775*
Drummond Island					-	0.336*	0.354*	0.288*	0.211*	0.191*	0.425*	0.258*	0.589*	0.747*	0.413*
Owen Sound						-	0.504*	0.403*	0.202*	0.238*	0.305*	0.438*	0.335*	0.831*	0.692*
Portage Point							-	0.065	0.156*	0.174*	0.559*	0.133*	0.594*	0.709*	0.552*
Waukegan								-	0.125*	0.061	0.424*	0.137*	0.499*	0.703*	0.418*
Old Mission									-	0.118*	0.301*	0.157*	0.270	0.448*	0.331*
Michigan City										-	0.290*	0.114*	0.445*	0.643*	0.175
Grindstone											-	0.543*	0.197	0.642*	0.079
Milwaukee												-	0.600	0.800*	0.620*
Parry Sound													-	0.503*	0.384*
Grand Isle														-	0.915*
Hamburg															-

Table 7. ANOSIM of fatty acid signatures among lake trout eggs across all sample sites in the phospholipid fraction of lipids. Values are R-statistics, which range between 0.0 and 1.0, with a value of 1.0 indicating that "within-group" samples are more similar to one another than they are to samples in another group, and a value of 0.0 indicating that the "within-group" samples are no more similar to one another than they are to samples in another group. "*" indicates a differences in FAS between sites.

Location	Taughannok Falls	Hamlin Beach	Clay Banks	Klondike Reef	Drummond Island	Owen Sound	Portage Point	Waukegan	Old Mission	Michigan City	Grindstone	Milwaukee	Parry Sound	Grand Isle	Hamburg
Taughannock Falls	-	0.469*	0.554*	0.836*	0.673*	0.785*	0.512*	0.508*	0.466*	0.498*	0.782*	0.535*	0.719*	0.570*	0.828*
Hamlin Beach		-	0.345*	0.702*	0.456*	0.696*	0.375*	0.300*	0.318*	0.247*	0.641*	0.478*	0.661*	0.370*	0.637*
Clay Banks			-	0.514*	0.222*	0.390*	0.083	0.094	0.125*	0.155*	0.458*	0.113*	0.499*	0.590*	0.661*
Klondike Reef				-	0.239	0.794*	0.571*	0.544*	0.205	0.600*	0.696*	0.781*	0.637*	0.820*	0.952*
Drummond Island					-	0.167	0.230*	0.410*	0.179*	0.300*	0.302*	0.313*	0.353*	0.450*	0.462*
Owen Sound						-	0.344*	0.568*	0.067	0.378*	0.334*	0.464*	0.303*	0.720*	0.700*
Portage Point							-	0.235*	0.124*	0.184*	0.474*	0.046	0.421*	0.480*	0.681*
Waukegan								-	0.242*	0.149*	0.530*	0.251*	0.626*	0.600*	0.641*
Old Mission									-	0.221*	0.217*	0.183*	0.043	0.120	0.283*
Michigan City										-	0.291*	0.176*	0.521*	0.440*	0.335*
Grindstone											-	0.594*	0.293*	0.650*	0.268
Milwaukee												-	0.572*	0.670*	0.810*
Parry Sound													-	0.470*	0.464*
Grand Isle														-	0.677*
Hamburg															-

Table 8. SIMPER routines of fatty acid signatures among lake trout eggs across all sample sites in the neutral lipid fraction of lipids. Values are percent dissimilarity between sample sites. The higher the number, the more different two sites are to one another.

Location	Taughannock Falls	Hamlin Beach	Clay Banks	Klondike Reef	Drummond Island	Owen Sound	Portage Point	Waukegan	Old Mission	Michigan City	Grindstone	Milwaukee	Parry Sound	Grand Isle	Hamburg
Taughannock Falls	-	7.16	9.79	10.60	9.96	10.18	9.98	9.80	9.18	9.75	12.17	8.96	11.30	8.79	10.32
Hamlin Beach		-	8.55	8.49	8.43	9.89	8.43	8.19	8.29	8.40	11.8	8.00	11.59	8.60	9.76
Clay Banks			-	8.86	7.92	9.08	8.68	8.09	8.72	8.38	10.67	7.29	11.18	11.55	9.53
Klondike Reef				-	8.14	9.75	9.40	8.82	9.41	9.14	11.65	8.41	11.63	10.66	10.09
Drummond Island					-	8.14	9.21	8.32	8.31	8.13	9.62	7.63	10.38	10.68	8.38
Owen Sound						-	10.94	9.52	8.86	8.96	9.00	8.48	9.04	10.23	8.76
Portage Point							-	8.63	9.34	9.27	12.89	8.08	12.97	12.54	11.66
Waukegan								-	8.94	8.53	11.42	7.59	11.83	11.73	10.11
Old Mission									-	8.96	10.73	8.06	10.53	10.32	9.86
Michigan City										-	10.4	7.81	11.35	11.54	8.93
Grindstone											-	10.5	9.99	11.15	8.10
Milwaukee												-	10.65	11.11	9.47
Parry Sound													-	9.87	10.12
Grand Isle														-	9.77
Hamburg															-

Table 9. SIMPER routines of fatty acid signatures among lake trout eggs across all sample sites in the phospholipid fraction of lipids. Values are percent dissimilarity between sample sites. The higher the number, the more different two sites are to one another.

Location	Taughannock Falls	Hamlin Beach	Clay Banks	Klondike Reef	Drummond Island	Owen Sound	Portage Point	Waukegan	Old Mission	Michigan City	Grindstone	Milwaukee	Parry Sound	Grand Isle	Hamburg
Taughannock Falls	-	6.68	7.20	8.9	8.67	8.23	7.12	7.14	8.17	7.02	8.78	6.37	8.53	6.44	8.78
Hamlin Beach		-	6.63	7.96	7.79	7.76	6.96	6.61	8.06	6.38	8.17	6.41	8.46	6.37	7.94
Clay Banks			-	7.53	6.94	6.64	6.05	6.23	7.38	6.27	7.54	5.50	7.80	7.50	8.48
Klondike Reef				-	7.06	7.72	7.54	8.09	8.39	7.82	9.03	7.41	8.85	7.55	8.94
Drummond Island					-	6.50	6.80	7.93	7.57	7.14	7.40	6.69	7.57	7.60	7.85
Owen Sound						-	6.54	7.78	7.15	6.67	6.41	5.93	6.22	6.75	6.83
Portage Point							-	6.73	7.01	6.39	7.46	5.43	7.29	7.07	8.33
Waukegan								-	8.19	6.42	8.20	5.99	8.93	7.95	8.73
Old Mission									-	7.60	8.20	6.77	7.60	7.51	8.68
Michigan City										-	6.82	5.72	7.80	6.90	6.86
Grindstone											-	6.94	7.32	7.79	6.59
Milwaukee												-	6.96	6.61	7.74
Parry Sound													-	6.99	7.83
Grand Isle														-	6.74
Hamburg															-

Table 10. Survival (%) of lake trout embryos to pigmented eyed stage, hatching stage, and swim-up stage in Taughannock Falls and Hamlin Beach in 2009 and 2010.

			Survival (%)		
Sample Site	Year	n	pigmented eyed stage	hatching stage	swim-up stage
Taughannock Falls	2009	24	81.4 ± 16.2	97.9 ± 2.7	75.0 ± 30.0
Hamlin Beach	2009	5	93.9 ± 0.2	68.5 ± 18.2	83.2 ± 5.9
Taughannock Falls	2010	26	85.9 ± 11.5	86.8 ± 20.2	92.4 ± 19.2
Hamlin Beach	2010	3	73.3 ± 26.0	91.7 ± 7.3	92.2 ± 3.6

Table 11. Pearson's coefficient (r) between survival of lake trout embryos to pigmented eyed stage, hatching stage, and swim-up stage and the concentrations of specific fatty acids in both the neutral lipids and phospholipid fractions of lake trout eggs from Taughannock Falls and Hamlin Beach (Data from 2009 and 2010 combined).

		Fatty acids in the NL fraction									
Location	Survival	15:0	17:0	18:0	18:3n-3	20:1	20:2n-6	20:3n-6	20:4n-6	22:4n-6	22:6n-3
	Pigmented Eyed	-	0.943	0.767	-	-	-	-	-	-	-
Hamlin Beach	Hatching	0.805	-	-	-	-0.816	-	-	-	-	-
	Swim-Up	-	-	-	-	-	-	-	-	-	-
	Pigmented Eyed	-	-	-	-0.298	-	-	-	-0.299	-	-
Taughannock Falls	Hatching	-	-	-	-	-	0.316	0.424	-	0.319	-
	Swim-Up	-	-	-	-	-0.373	-	-	-	-	-
		Fatty acids in the PL fraction									
		15:0	17:0	18:0	18:3n-3	20:1	20:2n-6	20:3n-6	20:4n-6	22:4n-6	22:6n-3
	Pigmented Eyed	-	-	-	-	-	-	-	-	-	-
Hamlin Beach	Hatch	-	-	-	0.708	-	-	-	-	-	-
	Swim-Up	0.873	-	-	-	-	-	-	-	-	-0.797
	Pigmented Eyed	-	-	-	-	-	-	-	-	-	-
Taughannock Falls	Hatching	-	-	-	-	-	0.368	-	-	-	-
	Swim-Up	-	-	-	-	-	-	-	-	-	-

Table 12. Primary prey item in each of the lake investigated and their major fatty acid. Fatty acid data were taken from Czesny *et al.* (2011). Note that fatty acid data comes from Lake Michigan prey fish and that the concentration of specific fatty acids of prey fish may differ among lake systems.

Lake	Primary Prey Item	Major Fatty Acid Associated with Prey
Ontario	Alewife	DHA
Erie	Rainbow Smelt	DHA
Michigan	Alewife/Round Goby	DHA/16:1n-7
Huron	Rainbow Smelt/Round Goby	DHA/16:1n-7
Superior	Lake Herring	Unknown
Champlain	Rainbow Smelt	DHA
Cayuga	Alewife	DHA

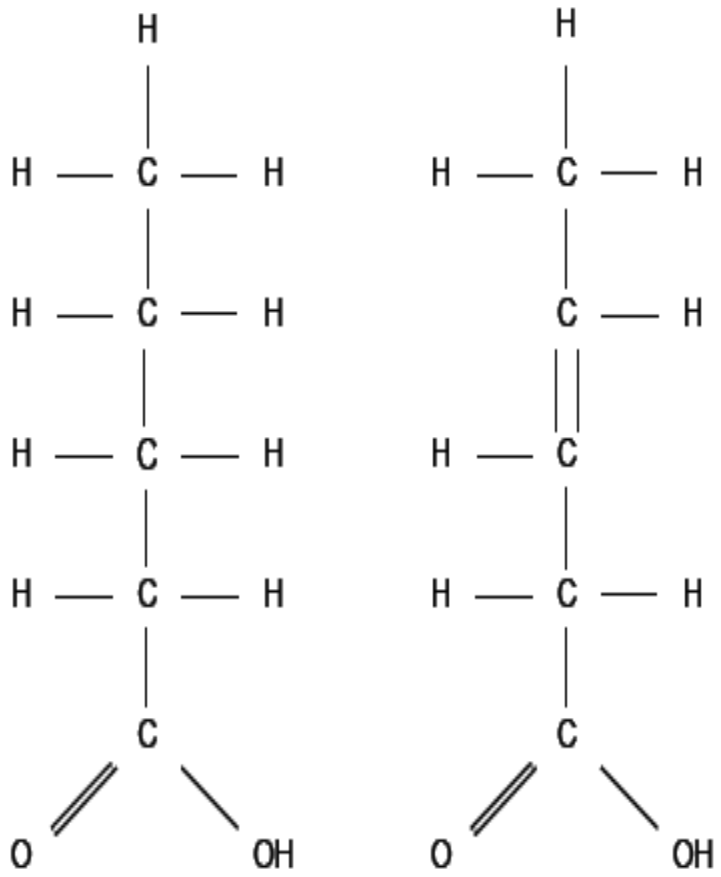

Figure 1. A saturated (left panel) fatty acid, with no double bonds in the hydrocarbon chain, and an unsaturated fatty acid (right panel), with one or more double bonds in the hydrocarbon chain.

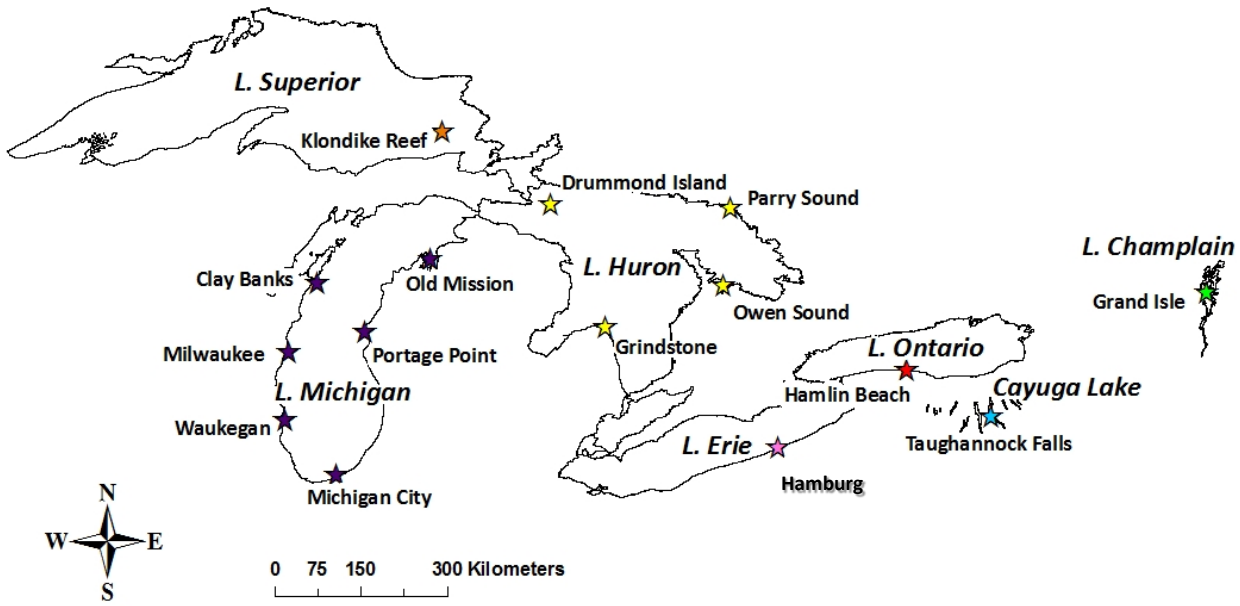

Figure 2. Study area: Great Lakes, Finger Lake (Cayuga Lake), and Lake Champlain. Stars indicate locations where lake trout eggs were sample for lipid and fatty acid analysis. Star color indicates lake.

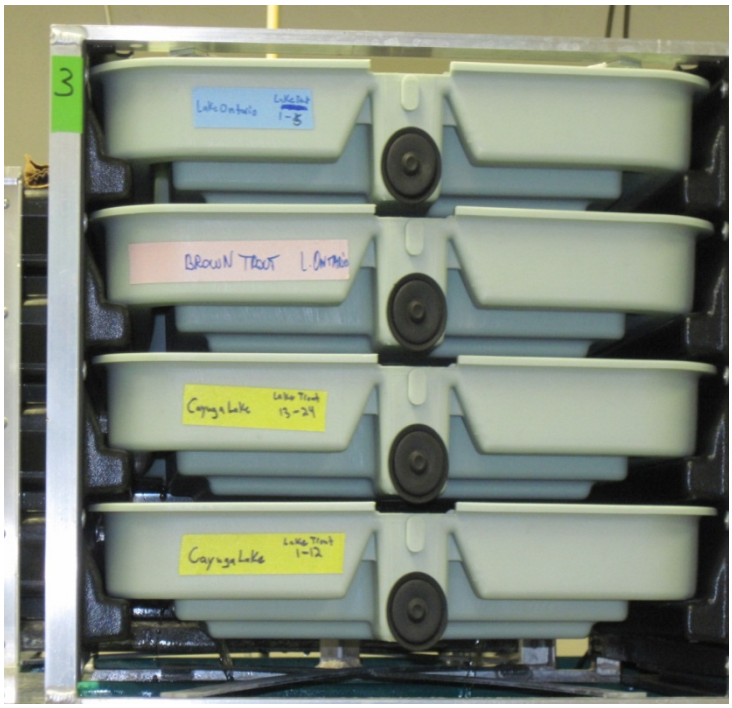

Figure 3. California hatching trays used for lake trout eggs incubation.

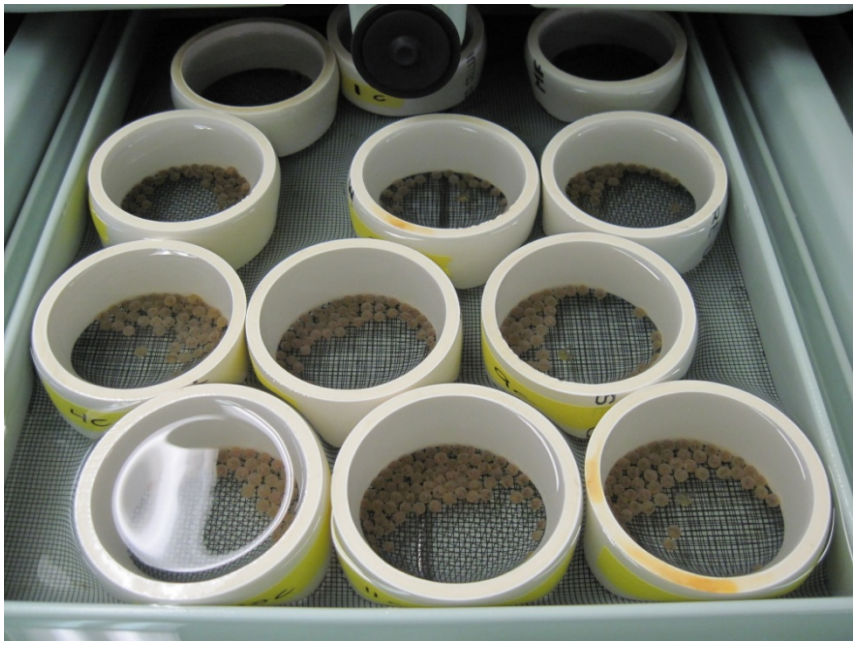

Figure 4. Individual tray holding mesh baskets. Each basket contains fertilized eggs from a specific female fish.

Figure 5. Aquaria used to determine alevin survival from hatching to swim-up stage. Each aquarium contains alevins from one specific female fish.

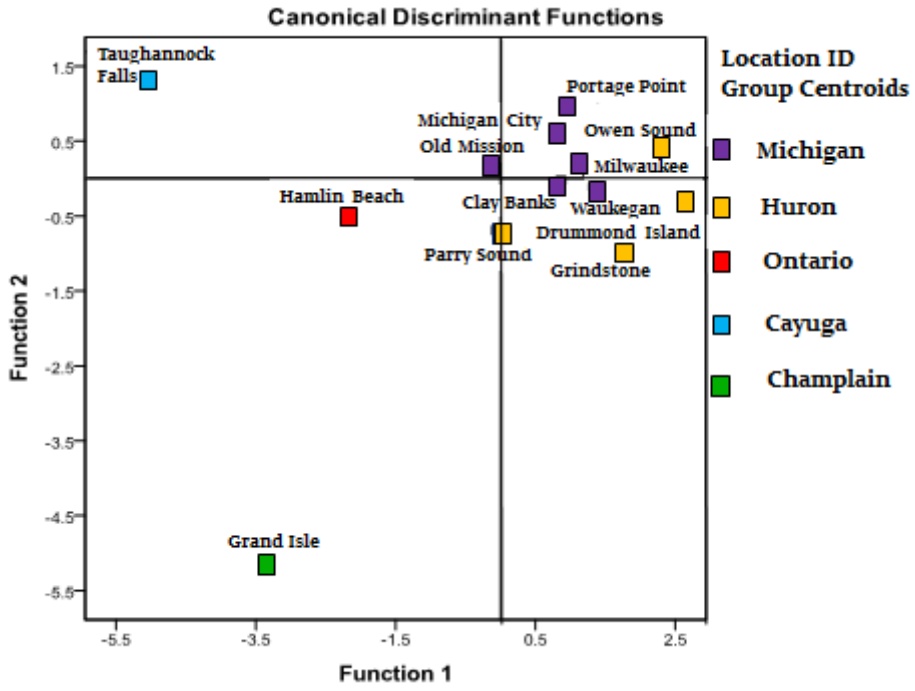

Figure 6. Discriminant analysis of lake trout eggs from 13 sample sites (with $n \geq 19$) using 18 fatty acids selected based on the largest variance of total fatty acids across all groups in the neutral lipid fraction of lipids. Plot shows the average scores of the first 2 of 12 discriminant functions that classified eggs to sample sites with a 77.7% success rate.

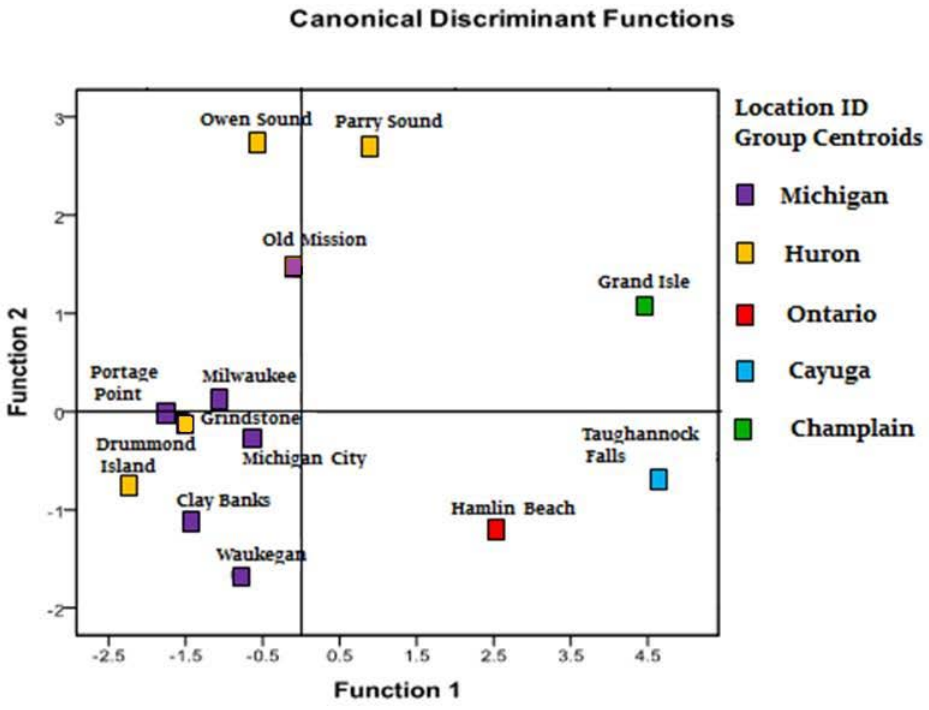

Figure 7. Discriminant analysis of lake trout eggs from 13 sample sites (with $n \geq 19$) using 18 fatty acids selected based on the largest variance of total fatty acids across all groups in the phospholipid fraction of lipids. Plot shows the average scores of the first 2 of the 12 discriminant functions that classified eggs to sample sites with a 77.3% success rate.

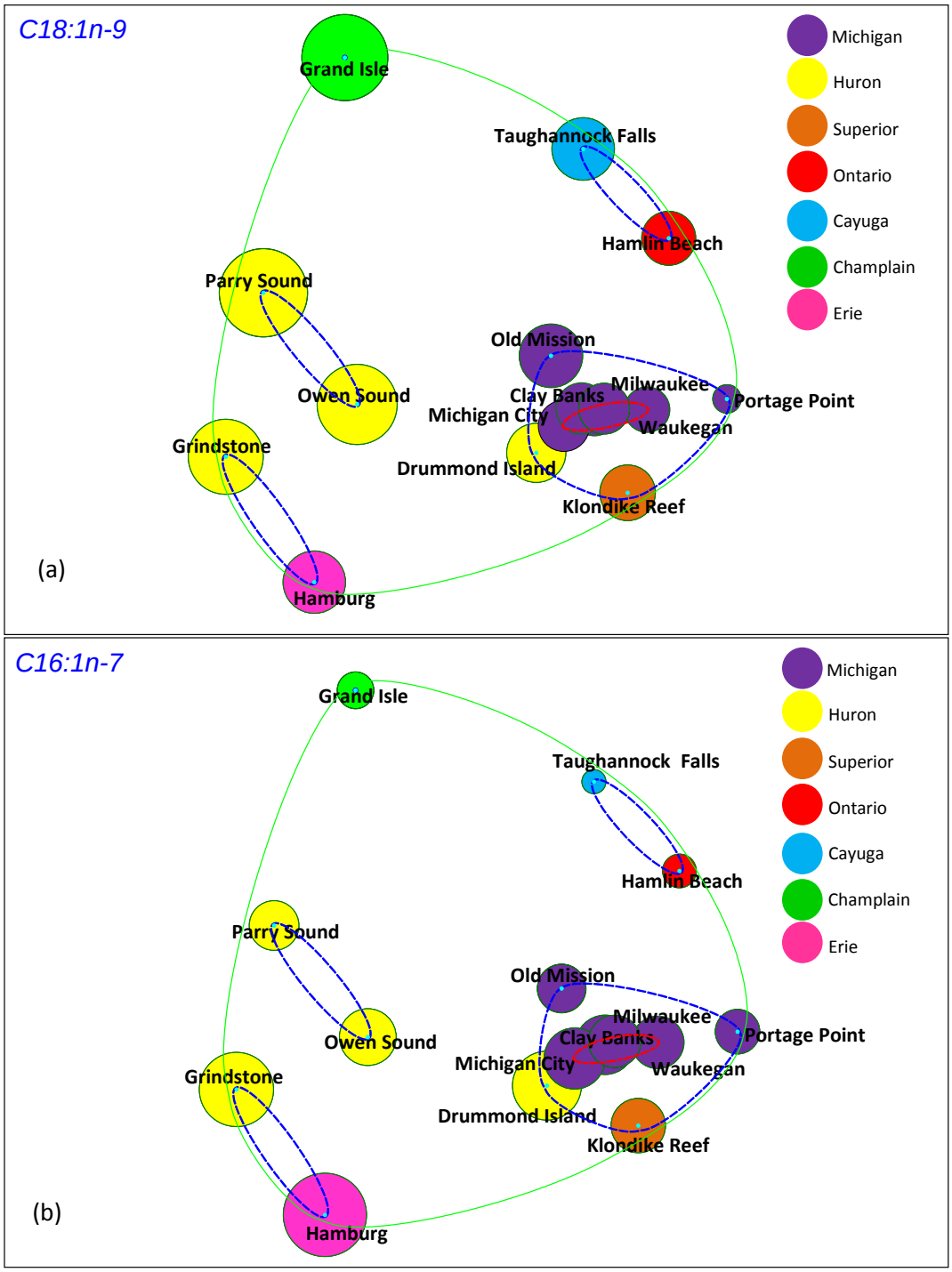

Figure 8. nMDS plot of group-averaged fatty acid signature data from all sample sites in the neutral lipid fraction of lipids. Contour lines from cluster analysis represent data groupings with specified similarity levels to aid in interpretation. Stress level 0.15. Solid green line represents 90% similarity. Large dashed blue line represents 95% similarity. Small dashed red line represents 97% similarity. Circles superimposed on data points used to represent relative proportions of fatty acids in data (a) 18:1n-9 (b) 16:1n-7 (c) 18:1n-7. Circle color indicates lake.

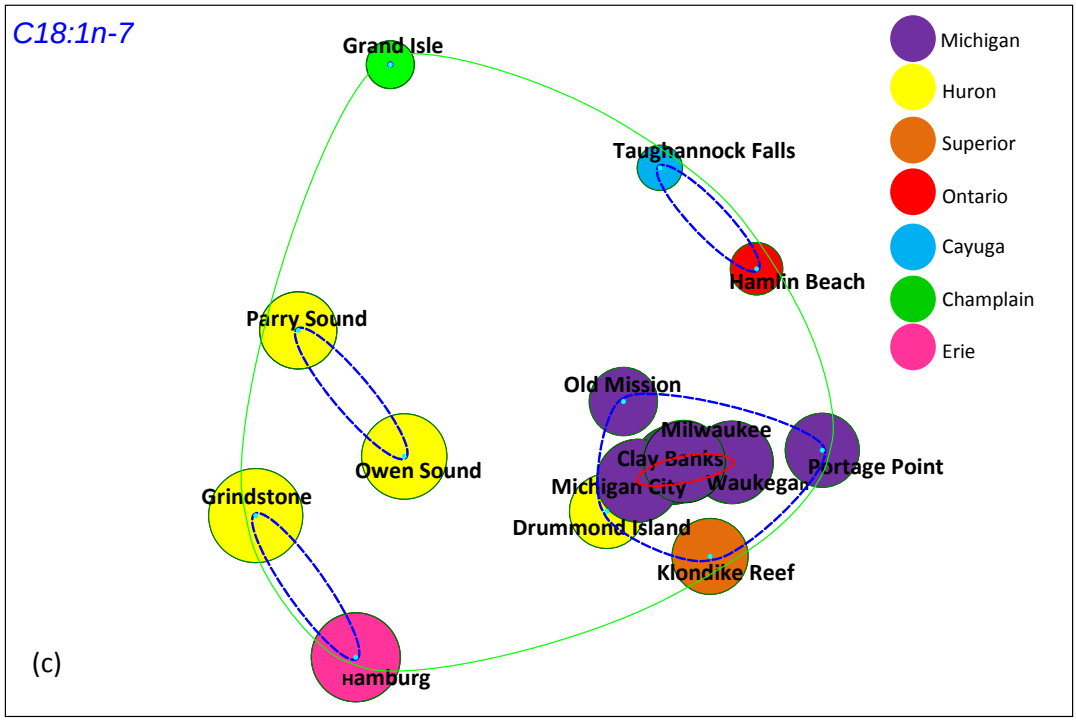

Figure 8. Continued from page 61. Graph (c).

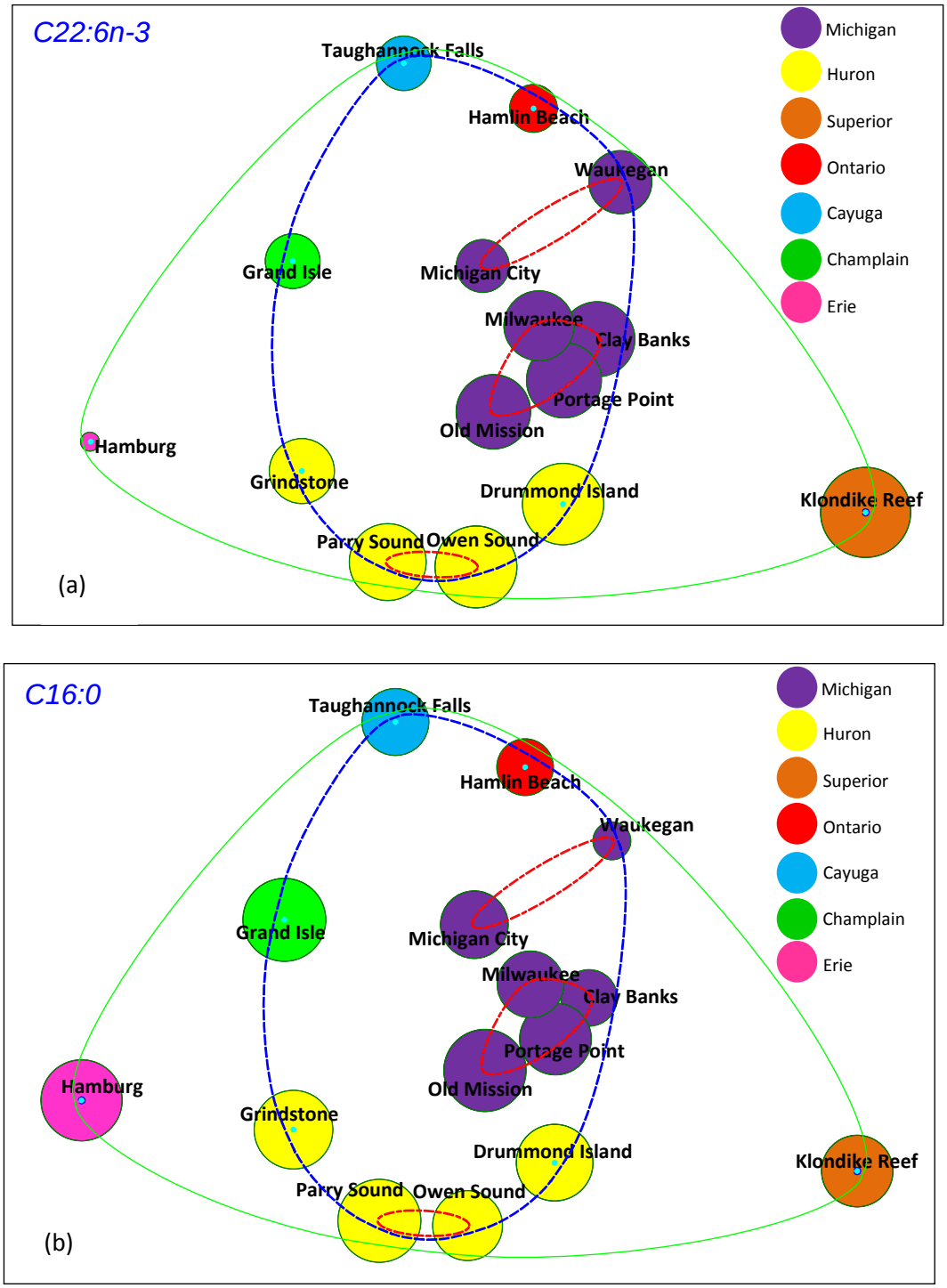

Figure 9. nMDS plot of group-averaged fatty acid signature data from all sample sites in the phospholipid fraction of lipids. Contour lines from cluster analysis represent data groupings with specified similarity levels to aid in interpretation. Stress level 0.15. Solid green line represents 90% similarity. Large dashed blue line represents 95% similarity. Small dashed red lines represent 97% similarity. Circles superimposed on data points used to represent relative proportions of fatty acids in data (a) 22:6n-3 (b) 16:0 (c) 20:4n-6. Circle color indicates lake.

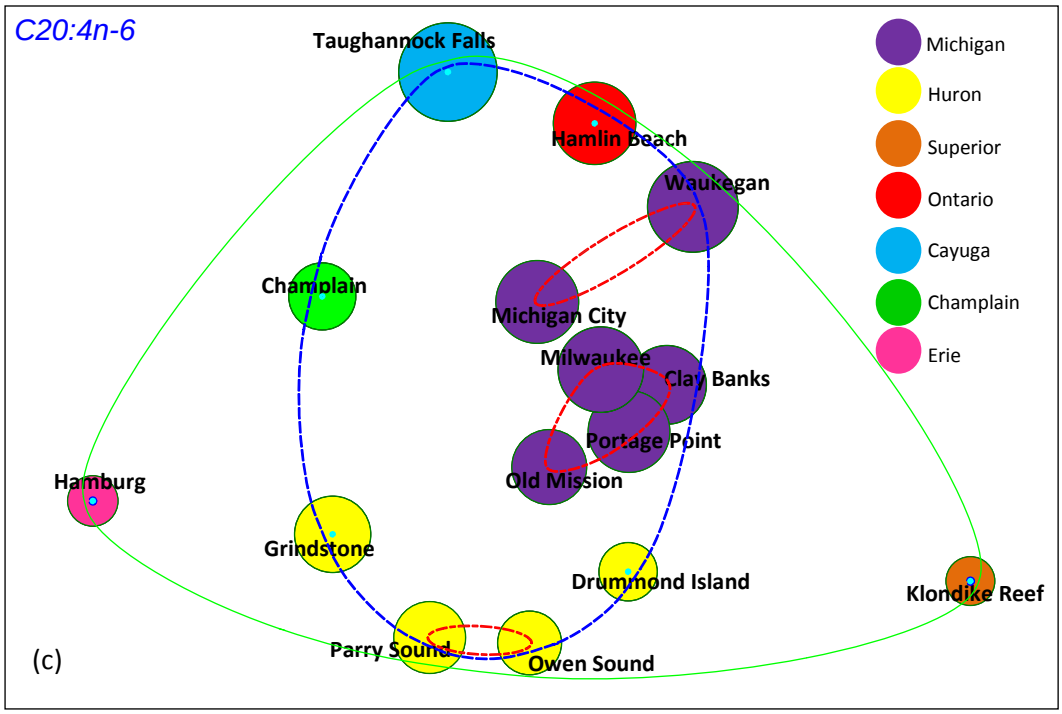

Figure 9. Continued from page 63. Graph (c).

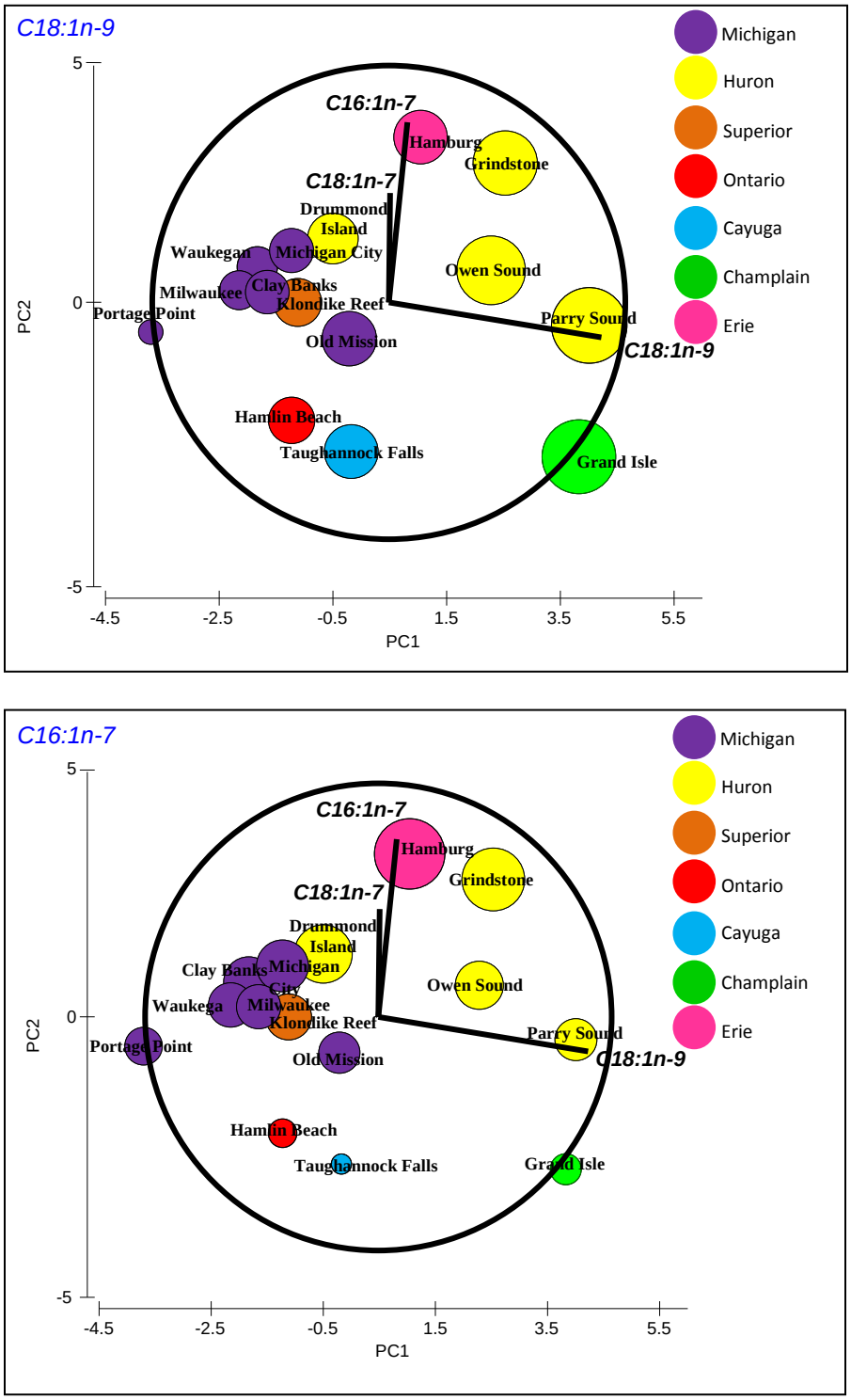

Figure 10. Plot of PC1 and PC2 (from PCA) of fatty acid signature data from all sample sites in the neutral lipid fraction of lipids. PC1 and 2 accounted for 78.8% of the variance in the data. 18:1n-9 is important on PC1. 16:1n-7 and 18:1n-7 are important on PC2. Selected correlations above 0.40. Circles superimposed on data points used to represent relative proportions of fatty acids in data (a) 18:1n-9 (b) 16:1n-7 (c) 18:1n-7. Circle color indicates lake.

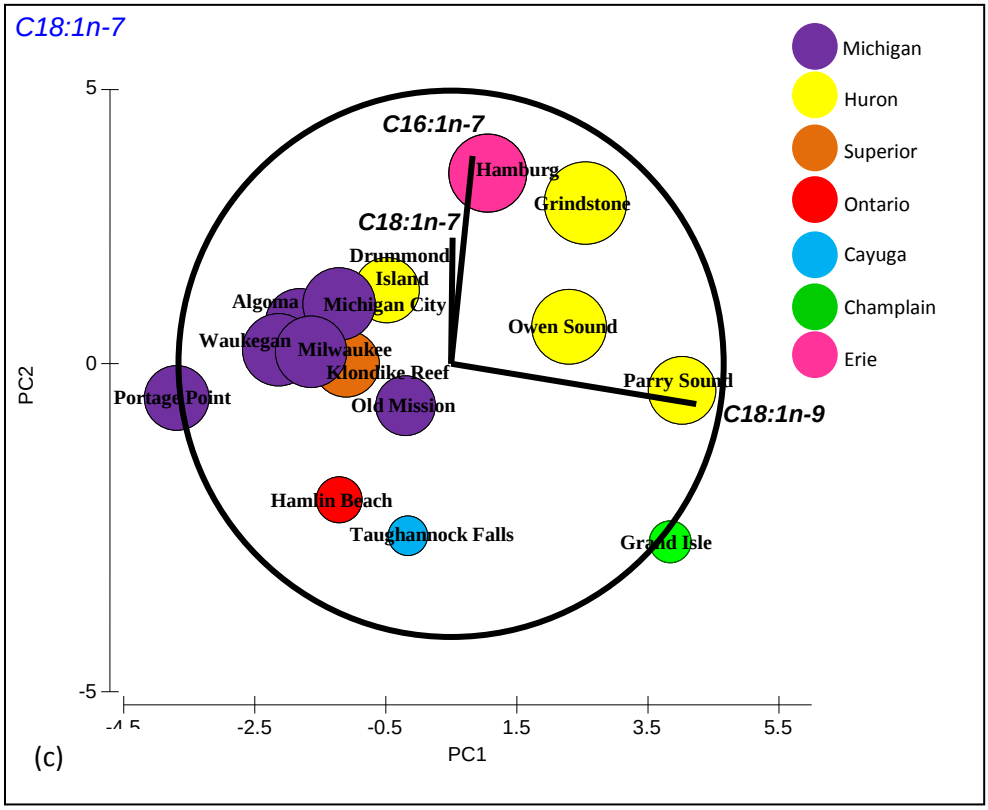

Figure 10. Continued from page 65. Graph (c).

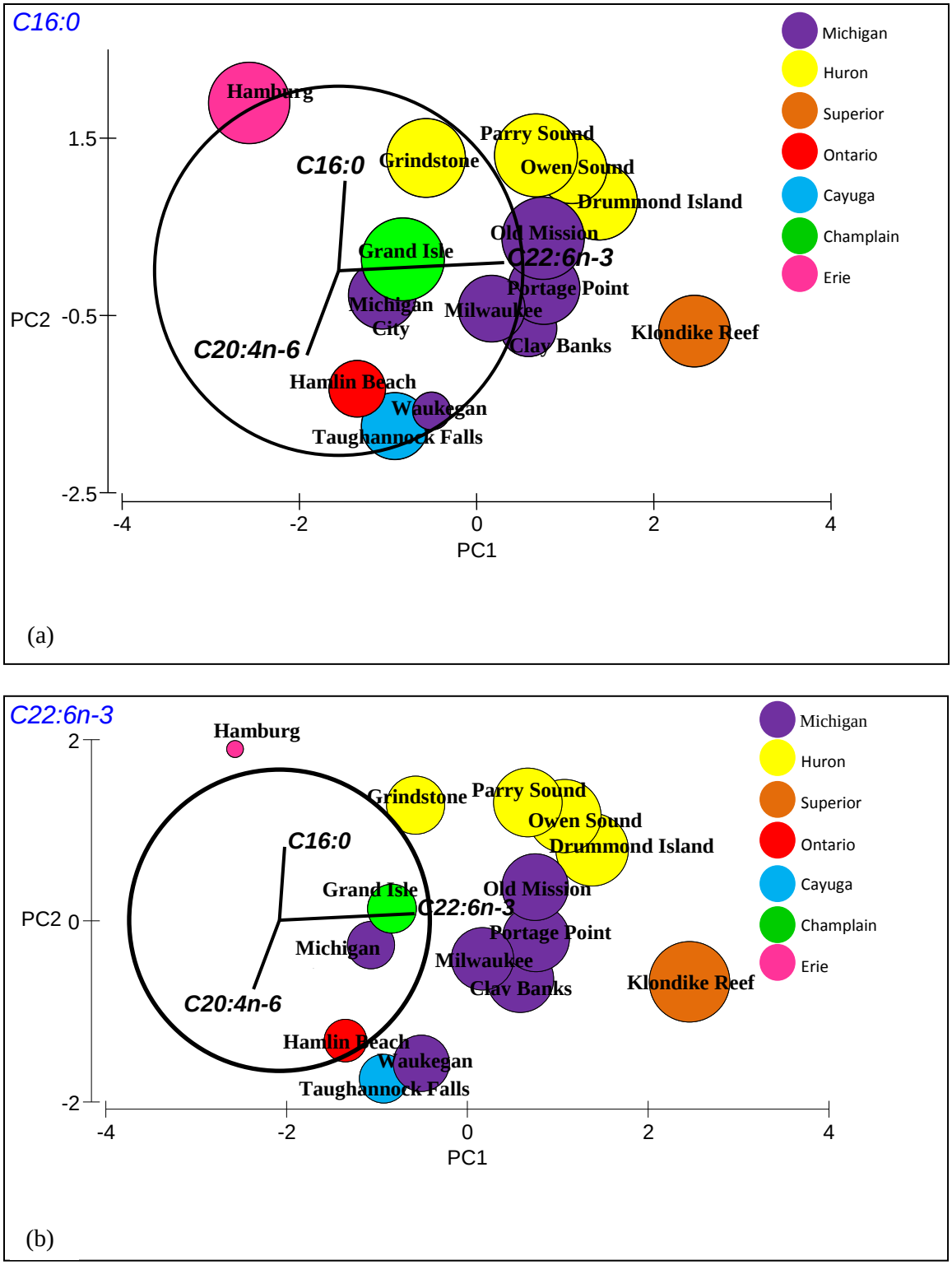

Figure 11. Plot of PC1 and PC2 (from PCA) of fatty acid signature data from all sample sites in the phospholipid fraction of lipids. PC1 and 2 accounted for 58.5% of the variance in the data. 22:6n-3 is important on PC1. 16:0 and 20:4n-6 are important on PC2.Selected correlations above 0.40. Circles superimposed on data points used to represent relative proportions of fatty acids in data (a) 16:0 (b) 22:6n-3 (c) 20:4n-6. Circle color indicates lake.

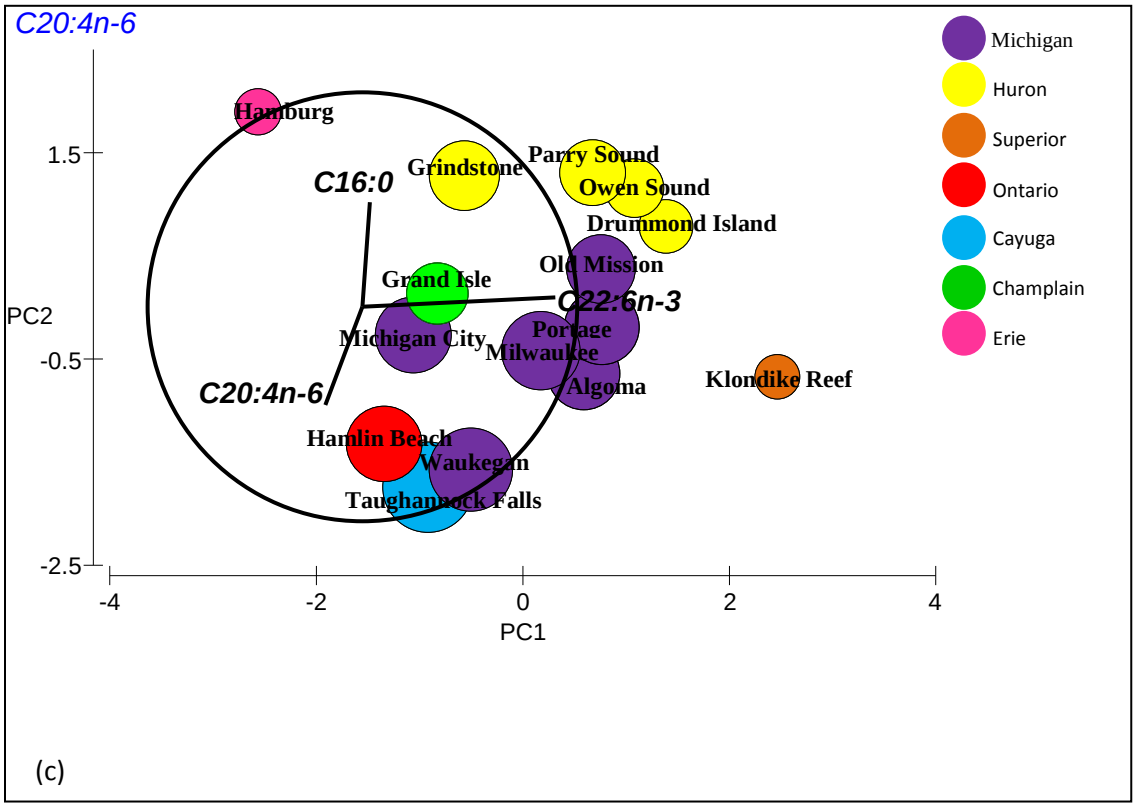

Figure 11. Continued from page 67. Graph (c).

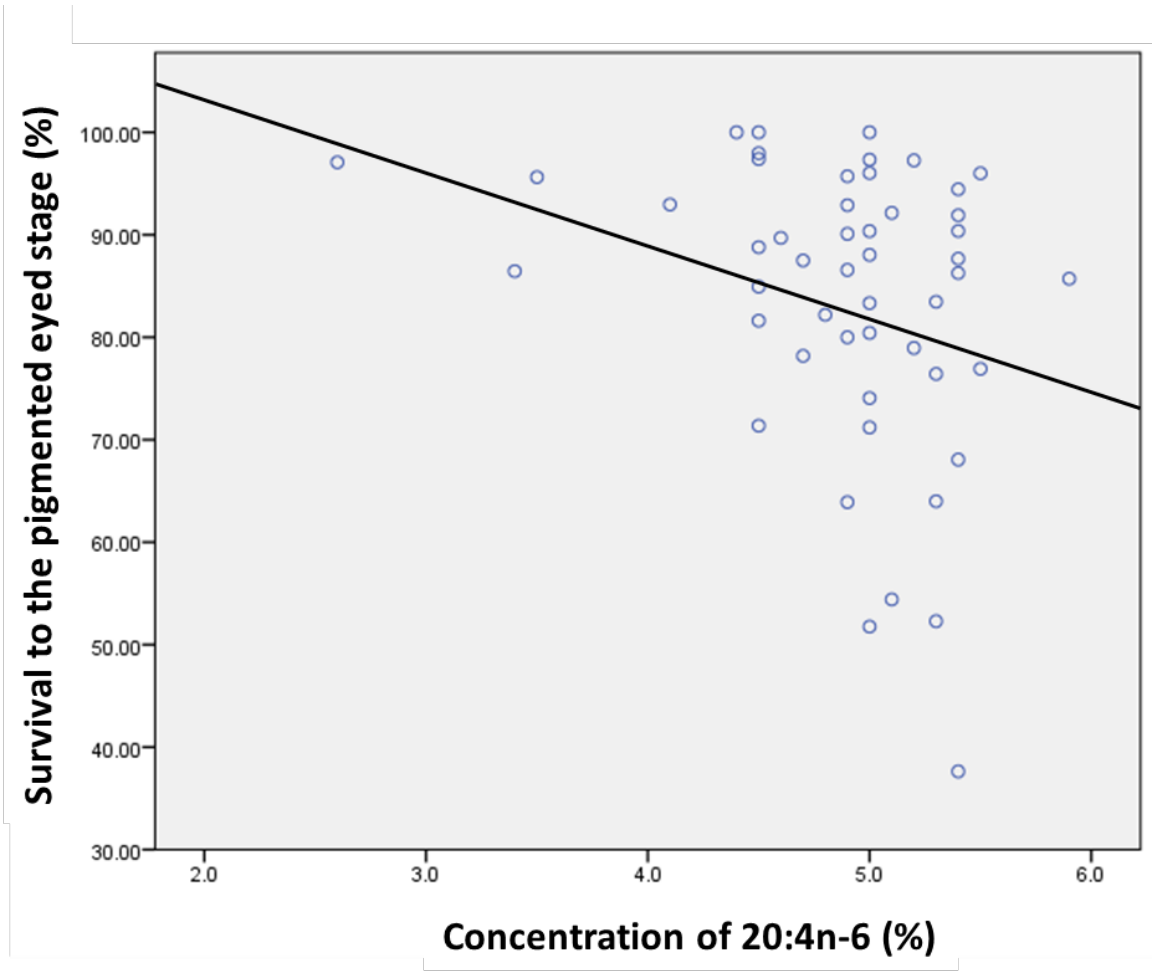

Figure 12. Relationship between embryo survival at the pigment eyed stage (%) and concentration (%) of arachidonic acid (20:4n-6) in egg neutral lipids (r = -0.229). Eggs were collected in Taughannock Falls in 2009 and 2010.

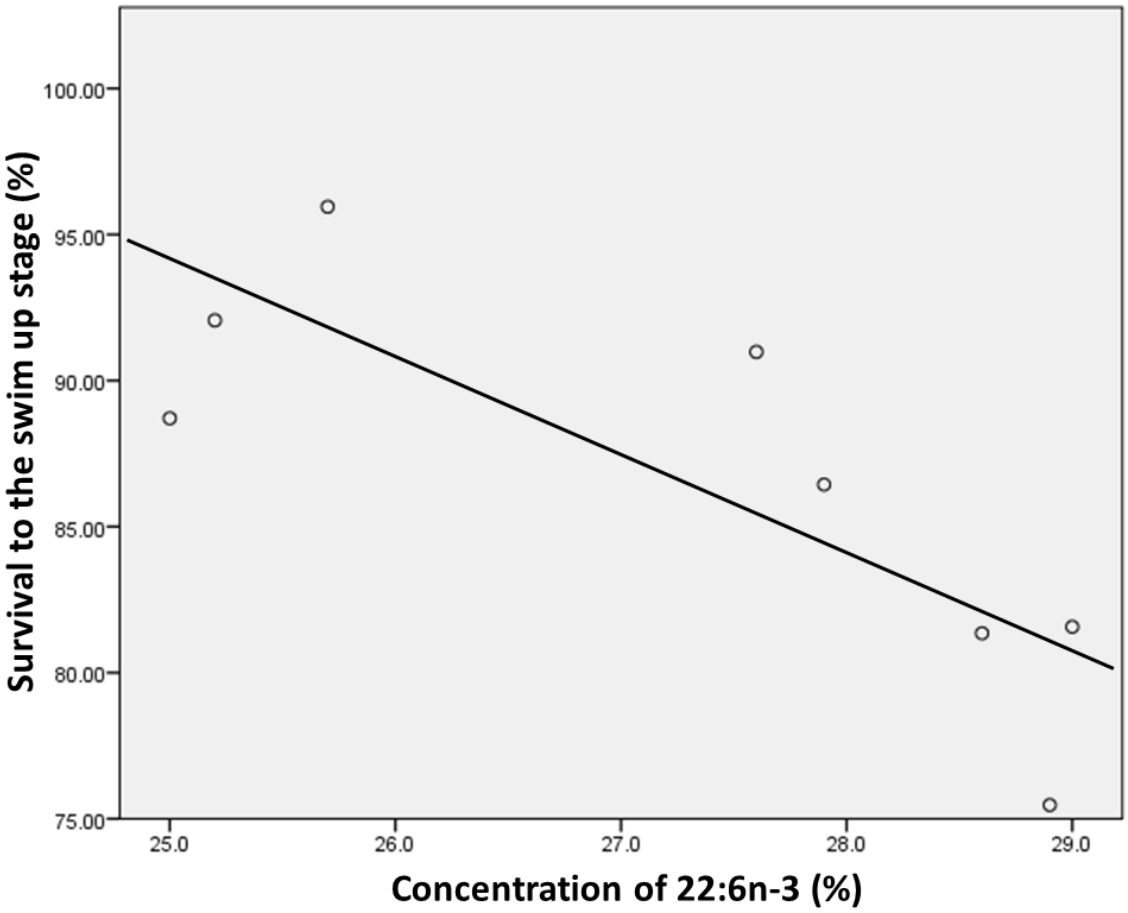

Figure 13. Relationship between embryo survival at the swim up stage (%) and concentration (%) of docosahexaenoic acid (22:6n-3) in egg phospholipids (r = -0.797). Eggs were collected in Hamlin Beach in 2009 and 2010.